Microbiology

A Fundamental Introduction

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Abstract

The paper is an introduction to microbiology, with emphasis on microscopy, bacterial structure, culture methods (enrichment, differential and selective), biochemical identification, serological methods, and an overview of Bacteriology, Mycology and Parasitology.

Keywords bacterial structure, microscopy, staining methods, culture methods, serology and fluorescent microscopy, darkfield microscopy, immunological methods and biochemical methods.

The World of Microbiology

I. Introduction

A. What is Microbiology?

Microbiology is the study of one-celled microscopic organisms. It deals with bacteria and other microorganisms that are from a few micrometers in size to as small as viruses which are the only 1 millionth of a millimeter. One of the largest viruses is the Pox virus, and is about 200 nanometers. Microbiology is not just the study of bacteria, but involves several other branches of biology.

i. **Protist, Monera and Procarotae**

Microorganisms are some of the smallest living creatures known to man. As such, it wasn't until Leeuwenhoek began to perfect the Art of grinding lenses, that it became possible for man to be able to visualize the worlds most elaborate and intricate creatures, "The Microbial World" [1].

Microorganisms are so extremely small, that without the aid of some magnifying device, they are virtually impossible to see. The average un-aided eye's vision is only capable of seeing objects separated by <200 nanometers). However, most microorganisms fall way below the resolving power of the human eye. Most microorganisms range in size between 50 micrometers, and on the average 1-5 micrometers (μ), with viruses around 25 nanometers [1-4].

In years past, microorganisms were originally assigned into large groups called Kingdoms. Many of these large groups had to be revised as new information became available. They were moved from one Kingdom to another, at times seemingly without a realistic scientific basis [5].

Haecke a German Zoologist in 1866 proposed grouping all microorganisms into a Third Kingdom Protista, and thus, lessing the confusion over classifying organisms with similar characteristics. At that time only the Animal Kingdom (Animalie), and the Plant Kingdom (Plantae) existed [5].

Margulis and Copeland proposed a four kingdom system in which the algae, protozoa, and fungi would be moved into the kingdom Protoclista. In 1969 Whitaker separated the Protoctista into two kingdoms Protista and Fungi. The bacteria and the blue-green algae (currently cyanobacteria) were placed into a separate Kingdom the Monera (also called Prokaryotae), thus again adding to the confusion. At last, in 1973 the Kingdom Prokaryolae was proposed, finally ending the confusion that had gone on for quite some time [5].

ii. **Kingdom Protista**

During that period there seemed to be much confusion on where to put the bacteria, and where in the world are we going to put the blue green algae, let alone those tiny tiny viruses? But at last the confusion was finally put to rest with the development of the Kingdom Protista. With the controversy over, microbiologists could now focus their interests upon the bacterial cell, its unique complexity and intricate metabolic processes. It also made clear the need for a closer examination between the prokaryotic cell, since eucaryotic cells are more compartmentalized with respect to their metabolic and genetic function [5].

The development of microscopes with greater resolving power, probably stimulated a greater interest in the comparing and contrasting the internal structures of both the Prokaryote and Eukaryote. These comparisons probably lead to the compartmental development of two types of cell systems, namely the Eucaryotic (true nucleus) and Procaryotic or primitive nucleus. Better staining techniques most likely contributed to changing to the two kingdoms system or Prokaryotes and Eukaryotes [6,7].

The Eukaryotic cells (Animal of cells) have a true nucleus (chromosomes) and membrane bound organelles. Prokaryotic cells, however, lack membrane bound organelles, as well as DNA in the form of distinct chromosomes. The need for the separation of bacteria into a separate kingdom became well established, and as such lead to a greater appreciation and understanding of the prokaryotes as a whole [6].

iii. **Eukaryote**

- **a.** Whitaker's 5 Kingdom Classification
- **b.** Microbial uses beneficial to Man
- **c.** Cell Reproduction

a. Whitaker's 5 Kingdom Classification

In 1969 Whitaker developed what was called the 5 Kingdoms, and the bacteria were thus relegated to the Kingdom Monera (which consisted of Eubacteria, Cyanobacteria and Archeobacteria). There was also the establishment of the Kingdom Protista (mostly single celled Eucaryotes), Kingdom Fungi (mostly multicellular molds, yeasts & macroscopic fungi), Kingdom Plantae (chlorophyll in chloroplasts), and the Kingdom Animalia (various eukaryotic animals) [5].

b. Microbial uses beneficial to Man

Although for the most part microorganisms have generally been given a bad image, there are only approximately one hundred genera that are pathogenic (disease causing). Of the thousands of other species that exist, the majority are beneficial to man. Many degrade and replenish biologically important materials, that otherwise would accumulate to massive proportions as waste. Fungi actively degrade dead plant debris and wood, whereas insects degrade other organic materials that would otherwise accumulate to enormous proportions. Soil microorganisms in symbiosis with some leguminous plants (clover) help capture atmospheric nitrogen, and replenish nitrogen back to the soil. Industrial applications have revolutionized the purity

and quantities of pharmaceuticals, which at no other time were possible to produce in such purity [8]. These genetically engineered microorganisms have led to the production of new antibiotics, growth hormones (ACTH), interferon, anticancer drugs and better disease treatment methods [8-10].

c. Cell reproduction

Reproduction as we know it, regardless of whether in plant, animal, prokaryotic or eukaryotic in general, involves cells multiplying by asexual reproduction, or cell division. Somatic cells of our bodies such as muscle, epidermal, etc., as well as plant cells reproduce asexually by cell division [11,12].

http://hucmportfolio.pbworks.com/f/Bacterial+Classification+and+Identification.pdf

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/E/Eubacteria.html

II. The Diversity of Microorganisms

A. Array of Microorganisms

B. Microbiology and its Branches

The World of Microbiology is a unique, ubiquitous array of one cell to multi-cellular microorganisms. Microbiology thus encompasses microorganisms that span the globe, and yet are even important to our own inner body wellness. In recent years, it has become apparent that microorganisms can affect how the body metabolisms drugs, as well as the way we respond to them. There may also be a microbe to microbe interactions within our intestinal tract. Even more significant is "the fact that changes in the intestinal commensal population have been correlated with many diseases in remote organs, such as in the case of diabetes, asthma, obesity, cancer, autism, and even depression [13,14].

In some cases, microorganisms can lessen the effects of drugs that may be toxigenic in the body. In a recent article by Antunes et al. entitled the "Mining Bacterial Small Molecules," our intestinal flora produce a multitude of small molecules (steroidal), and chemical messengers that can affect distant organs, and in some cases modulate metabolism, as well as the immune system. Thus, the loss of our own normal flora can severely upset the body's homeostasis, and make us more susceptible to disease [13,14].

Many opportunistic flora of bacteria and yeasts that roam within our intestinal tract are usually held in track by our numerous normal intestinal flora. However, in instances where our normal flora are wiped out during antibiotic therapy, or other invasive procedures, other endogenous microorganisms may multiply rapidly, and can cause secondary infection. These opportunistic microorganisms can colonize in higher than normal numbers, and thus bring on the onset of secondary disease [7,15,16].

Our normal flora help keep in check those commensal microorganisms that would otherwise overgrow, and then cause an infection. However, under times of lower resistance, they can colonize our bodies to the extent of disease [16]. Many A.I.D.'s patients because of their immunocompromised state, tend to be over-run by the parasitic microorganism *Pneumocytis carinii,* as well as many types of Mycobacteria (Mycobactera avium intracellulare Complex). This is particularly apparent in those individuals that are compromised, because of the presence of diseases such as in the case of cystic fibrosis, AIDs patients, the elderly, dialysis patients, cancer, and others predisposed for some other reason. The presence of our own normal flora therefore can be particularly important, since they prevent other usually insignificant microorganisms from over growing and causing disease [10,16-18].

B. **Archaea**

Microorganisms can be found in just about any environment that one can think of a period. Archae for example live in some of the most inhabitable parts of our world. Some Archaea have been found in temperatures as high as 98 °C, in the hot water geysers in Yellowstone Park and Purple Sulfur bacteria) [19-21].

Archaea have also been found to thrive even at depths on the ocean floor, at of thousands of feet below. These microorganisms are able to resist temperatures that would fry you or me. They have even been found at the bottom of the ocean near Tectonic plates "tube worms" [21], Geysers of Yellowstone [20], and the Antarctica [21]. Other Archaea found in Antarctica may be found (Cyanobacteria) as psychrophiles living within the blue layer of the permafrost, between the cold loving fungi and bacteria [22,23]. They are able to survive the harsh environment of the Antarctic, by living just below the surface of the rocks [19-23].

Another group of microorganisms are able to grow in another extreme environment, namely the Carlsbad Caverns National Park. These organisms are thermoacidophiles, and they live within the caverns surviving not only the high temperatures, but also the presence of high levels of hydrogen sulfide (H_2S). They are located in the Carlsbad Caverns National Park, and are often referred to as Sulfur bacteria that oxidize H_2S and form "Snottites" [8,24]. They are bacteria that oxidize Hydrogen sulfide (H_2S) that is abundant in the air, and in process form sulfuric acid. As the colonies grow in mass they form a mucus-like string of colonies, that release drops of sulfuric acid as they grow [20,24].

By contrast the thermophiles in the Gold mine near Johannesburg, Africa (*Bacillus infernus*) survive temperatures of 200 °F, and are highly unique, in that they are "powered by the radioactive decay of unstable uranium atoms in the rocks [25].

Other unique examples of microorganisms would include *Deinococcus radiodurans* which can survive one thousand times the level of radiation that would be lethal to the average man [26]. Whereas the genus *Halococcus* can be found in the Great Lakes of Utah, and can live in salt concentrations up to 30% [26,7].

In 1977, one of the most unique and intriguing ecological systems was discovered off the coast of the Galapagos Islands. At a depth of approximately 2650 meters and 662 °F, Marine biologists were able to explore a unique ecosystem deep in the ocean. What they found was a unique symbiotic relationship between bacteria, and tube worms near the hyper-thermal vents. In a process called "chemosynthesis"; the bacteria are able to use the chemical energy in the form of inorganic compounds (H_2S), to convert carbon dioxide into the organic molecules that make up carbohydrates and sugars [21,26,27].

It has been found that the tube worms concentrate hydrogen sulfide in their blood, with hydride as the central element of their hemoglobin instead of iron which is normally found in human hemoglobin. The bacteria thrive on the presence of hydrogen sulfide, and the absorbed CO_2 from the tube worms, which is used as a carbon source in manufacturing foodstuffs needed by the tube worm [21].

C. **The Procaryotes** [1-3,5,28]**.**

1. **Parasitology:** Study of worms, helminths, protozoans and amoeba.
2. **Immunology:** Study of the Immune system and how the body protects itself from disease.
3. **Mycology:** Study of fungi (medical, food & environmental).
4. **Phycology:** Study of algae (blue, green, red & brown) [3,4].
5. **Virology:** Study of viruses (contain either RNA or DNA but not both and a protein coat).
6. **Bacteriology:** Study of bacteria, various genera [4].

http://textbookofbacteriology.net/procaryotes.html

http://classes.midlandstech.com/carterp/Courses/bio225/chap10/lecture1.htm

Classification--hierarchy

http://plantphys.info/organismal/lechtml/bacteria.shtml#cell

III. Classification of Bacteria

Although microbiology is primarily concerned with the study of microorganisms. It does draw much from the all these branches other branches, namely: Cell Biology, Biochemistry, Chemistry, Microscopy, Physiology, Immunology, Metabolism and Genetics. All of which provide an integrated whole and credence for the accumulation of information for microbiology [4].

In 1990 Carl Woese and G.E. Fox proposed the establishment of a new level of classification above the kingdom level, what was called the domain system. In his 3 domain system the bacteria and archae would gain domain status. In the Domain system of classification the Prokaryotes (previous method by Bergey's manual) would belong to the Domain Bacteria and Archaea. They had previously been classified in the Kingdom Monera, which included the Phyla Eubacteria, Cyanobacteria and Archaeobacteria [5]. The Cyanobacteria were formerly known as the blue-green algae, because of being photosynthetic, unicellular, heterotrophic and with the ability to fix atmospheric nitrogen, were also placed in the domain Archaea [5,29,30].

http://www.buzzle.com/articles/characteristics-of-archaebacteria.html

http://hucmportfolio.pbworks.com/f/Bacterial+Classification+and+Identification.pdf

I. Different Archaea and Other Phyla

A. Extremophiles

1. Methylogens-reduce carbon dioxide containing compounds to form the as methane.
2. Extreme halophiles- live in excessively salty environments (Halococcus species).
3. Thermoacidophiles- live in hot acid environments, such as volcanic vents in the ocean floor. They also are found at the deep ocean floor, where hot volcanic vents release toxic gas and are extreme temperatures.

B. Protista are eukaryotic microorganisms that have a true membrane bound organelles within the cytoplasm, and include the algae, euglenoids and Photoplankton.

C. Fungi are eukaryotes that lack true leaves, roots or stems and contain chitin or cellulose in their cell walls. Some fungi have sterol in their cell membrane [31,32].

D. Animalia includes all animals derived "from a zygote a cell formed by the "union of two gametes such as egg & sperm,". Parasites are eukaryotic worms that parasitize humans and are the Helminths which include flukes (trematodes), tapeworms (cestodes), and roundworms (nematodes) which live inside the body of their host [5,31,33,34].

E. Bacteria

Bacteria are one celled microscopic organisms that generally reproduce by binary fission. One of the unique things of bacteria not commonly found in animal cells, lies in the outer most covering - the cell wall. Instead of a plasma membrane alone, they have an outer cell covering, the cell wall. The cell wall is mainly composed of a particular disaccharide (Carbohydrate). Not only does the cell wall protect's the bacteria from its environment, but it also provides shape and rigidity to the cell [6].

http://plantphys.info/organismal/lechtml/bacteria.shtml

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/E/Eubacteria.html

http://plantphys.info/organismal/lechtml/bacteria.shtml#cell

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/E/Eubacteria.html

Classification of Bacteria: Bacteria are classified based on the size, shape (whether rod shaped, spherical and spiral shaped), types of motility (presence of or absence of motility methods-flagella), the type of cell wall present, biochemical characteristics, biochemical capabilities, serological reactions, as well as their genetic characteristics. Bacteria may have several shapes such as bacilli (rod shaped), cocci (spherical), helical (spiral shaped), and even star shaped. The cell wall is not only important in regulating the entrance of substances in and out of the cell, but also is important in antibody reactions, and immunological reactions as well. Biochemical tests using the cell wall antigen have historically been used in laboratories for identification purposes for years, and is still are used in many microbiology labs [5,6,35,36].

http://www.scf.edu/pages/PDF/NaturalSciences/BlackMicrobiology6e-Chapter9.pdf

http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookDiversity_2.html

Bacterial Structures: There are a number of structures that make up bacterial cells. The cell wall is important in that it protects the cell contents from being leaked, and is involved in protection from hazardous chemicals. It is also antigenic, meaning it can elicit antibody formation. Another aspect of the bacterial cell wall is that it contains a "unique compound," namely the "peptidoglycan" (Special Carbohydrate). The peptidoglycan is uniquely found in various types of bacteria with the exception of the genus Mycoplasma, Ureaplasm, Rickettsia which lack cell walls [6,7,30,34,37].

http://www.atsu.edu/faculty/chamberlain/Website/Lects/RICKETT.HTM

http://www.microbiologytext.com/index.php?module=Book&func=displayarticle&art_id=60

http://bioserv.fiu.edu/~walterm/gen_bio_II/sum10_lecture1a_prokaryotes_small.pdf

III. Classification of Bacteria continues

A. Structures of Bacteria

i. **Cell Wall**: Protects inside contents of cells: contains peptidoglycan and some lipids (fats).

ii. **Cell Membrane:** Contains enzymes for cellular respiration, murein and peptidoglycan synthesis, oxidative phosphorylation as well as transport of substances in and out of the cell.

iii. **Cytoplasm:** It is an amorphous gel containing enzymes, ions & various small structures similar to organelles that contain structures like cytoplasmic inclusions.

iv. **Mesosome:** generates energy as ATP (like mitochondria of the cell). "Now considered microscopic artifacts" by some researchers.

v. **Ribosomes:** proteins are synthesized on them 70 S (swedburg unit).

vi. **Nucleoid:** is not a true nucleus, and is called the genophore, (nucleus like body). It is where there is a concentration of genetic material or DNA.

vii. **Enzymes:** special proteins that act as catalysts, and help biochemical reactions to occur [7,28,30,38,39].

http://www2.estrellamountain.edu/faculty/farabee/BIOBK/BioBookDiversity_2.html

Photographs on Bacterial Structure, first listed

http://www.textbookofbacteriology.net/structure.html

http://www2.estrellamountain.edu/faculty/farabee/BIOBK/BioBookDiversity_2.html

B. Accessory Structures

Appendages

1. Glycocalyx: is a viscous gelatinous polymer, that is external to the cell wall and composed of polysaccharides, polypeptide or both.
2. Endospores: Resting cells capable of withstanding harsh conditions such as high temperatures, and pressure.
3. Flagella: Long whiplike or "threadlike" appendages used to propel or for the microorganism to swim.
4. Capsule: Polysaccharide coat: a sugar-like coat or covering that helps protect microorganisms from being phagocytized by host cell defenses (Immunity).
5. Pigments: may be different colors such as reds, blues or purple, and that are imparted to bacteria as they grow on culture media, generally polysaccharide (carbohydrate). Some pigments are used in the manufacture of food in special Phototrophic bacteria, they use ultra-violet light to make food [6,7,11,30].

http://www.textbookofbacteriology.net/structure.html

http://bioserv.fiu.edu/~walterm/gen_bio_II/sum10_lecture1a_prokaryotes_small.pdf

http://plantphys.info/organismal/lechtml/bacteria.shtml

http://hucmportfolio.pbworks.com/f/Bacterial+Classification+and+Identification.pdf

Observing Bacteria

Wet Mount -The inoculum is emulsified in water and is observed on a slide under the microscope. This type of observation can tell little about the size and shape (morphology) of the microorganisms. Microorganisms are difficult to observe without the aid of a microscope.**Staining:** Dyes stain organisms making them more visible under the microscope. This way the size, shape and other characteristics can be seen [2,40].

http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/gram.htm

http://www.eplantscience.com/index/biotechnology_methods/microbiology/bacterial_staining.php

http://www.google.com/l?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CCEQFjAA&url=http%3A%2F%2Fscience.kennesaw.du%2F~jhendrix%2Fbio3340%2Fhandouts%2Fmicrotaxonomy.ppt&ei=O76FVdCRMoemgwSSzYDYDg&usg=AFQjCNE2UKwC7De8s4imabiLlXp-esmfcA&bvm=bv.96339352,d.eXY

Microbial Taxonomy & Classification

Wet Mount & Staining Methods

Special Microscopes: With Fluorescent and Electron Microscopes, One can see much detail, since the magnification is much greater than the light microscope.

Fluorescent Microscopes: Microorganisms are tagged with fluorescent dyes or fluors.

Fluorescent dyes: Organisms glow a bright color, usually olive green when exposed to ultraviolet light (UV).

The microorganisms: Thus can be seen under this microscope, since the dye glows or fluorescences under uv light [2,7,40,41].

Therefore, with tagged antibodies (abs) equal: (Abs + fluorescent dye) + uv light = bacteria glow green under uv light [2,40,41].

4. **Serological tests:** Animals are injected with bacterial preparations at certain intervals, usually at 7 and 14 days. The animals are bled, and the blood is spun in a centrifuge, and the liquid (serum) portion is saved [29,42].

The blood cells are thus packed at the bottom and the liquid plasma is saved, since it contains the desired antibodies. In this way animals are inoculated with specific bacterial types, and blood is removed at intervals. The test animal will react to the foreign antigen, and will begin to produce antibodies that should be found in its blood serum [42].

What is interesting is the fact that any time the antisera comes in contact with the bacteria that stimulated its formation, it will agglutinate (clump). They cause the bacteria to clump Together (agglutinate) on a slide, and this concept can be used to detect that bacterium. Because the antibodies formed by the rabbit, in its serum are specific for that particular organism that stimulated their formation [42,43]. Thus, these serological tests may be used for the detection of antibodies or antigens that react only with the specific bacterial antigen that stimulated their formation [29,42,43].

http://uhaweb.hartford.edu/BUGL/immune.htm

http://classes.midlandstech.com/carterp/Courses/bio225/chap10/lecture3.htm

http://www.bing.com/videos/

Immune System

http://www.bing.com/videos/search?q=Serological+Techniques

Staining Methods

Routine Staining With Dyes

Most important is the Gram stain, invented in 1884 by Christian Gram [2]. Gram found that by using certain aniline dyes the microorganisms could be more readily seen under the microscope [2,40].

http://www.bing.com/images/

Gram Stain Procedure

1. After a smear is prepared on the slice, the slide is next heat fixed and then the 1st stain is applied and timed.
2. Crystal Violet (CV): Stain 1 minute
3. Gram's Iodine: Stain 1 minute
4. Decolorize: With alcohol till colors out
5. Safranin: Stain 1 minute [2,40].

If a bacterial cell contains high levels of the peptidoglycan in its cell wall, it will tend to retain the Crystal violet within the cell wall, and will appear purple after the addition of crystal violet and Gram's Iodine. However, if a microorganism contains only a small amount of peptidoglycan, and fairly high levels of fats or Lipid materials, they have a tendency to be decolorized by the addition of alcohol. They will stain red with the counterstain safranin. Whereas those that retain the crystal violet stain appear purple when observed microscopically, and are said to be gram positive, while those that are decolorized by alcohol and retain the safranin, are considered gram negative. Another staining method that is important would include the acidfast stain. This stain is used to recognize the genus Mycobacterium and Nocardia which are both acidfast [2,44,45].

http://microbeonline.com/types-of-staining-techniques-used-in-microbiology-and-their-applications/

http://www.eplantscience.com/index/biotechnology_methods/microbiology/bacterial_staining.php

Importance of Gram Stain

The ability to separate bacteria into two groups is based on the gram stain and consequently lead to the realization that if chemical agents could be added to culture media, not only would microorganisms be isolated, and would be selected based on their propensity to certain dyes and chemical agents, as well as the type and quantity of selecting agents formulated [2,11,40,41].

Special Stains

Flagella, Capsule and Endospore Stains

The addition of dyes and or antibiotics added to culture media can specifically formulated to inhibit microorganisms, while allowing the growth of other group microorganisms. For example the culture medium eosin methylene blue agar, will thus allow only gram negative microorganisms to grow, because the addition of eosin and methylene blue inhibits gram positive, but allows the gram negative bacteria to grow or vice versa [2,11,12,40,41,46,47].

A variety of bacteria may be stained by gram stain, or for acidfast, flagella, and capsule stain [2,7,11,12,40,41]. For example, using Bismuth Sulphite Agar (BSA Agar), the dye brilliant green and bismuth sulfate are incorporated into agar, in order to inhibit gram positive organisms, but gram negative organisms are not affected by the dye. So the gram negative microorganisms are able to grow. This culture medium is selective for Salmonella, and retards the growth of coliform bacteria. Many bacteria which grow on culture media may be selected or inhibited based on the addition of dyes and selective agents [2,11,40,41,47].

http://delrio.dcccd.edu/jreynolds/microbiology/2421/lab_manual/atlas/biol2420photoatlas_002.htm

Reproduction

1. Reproduction is by binary fission- cells split into new cells
2. Conjugation is the transfer of DNA from a donor bacterium to a recipient bacterial cell.
3. Special DNA: plasmids are small circular units of DNA that replicate (divide) independently from the DNA found in their

primitive nucleus. Some organisms may have several plasmids [28,30,48,49].

http://classes.midlandstech.com/carterp/Courses/bio225/chap10/lecture3.htm

Bacterial Genetics

For example, Corynebacterium diphtheria causes the disease Diphtheria. This microorganism can only cause disease when it has been infected itself by a special virus called a Prophage. When the virus infects a bacterium instead of killing the bacterium, the viral DNA (prophage) becomes part of the circular bacterial DNA and providing the instructions allowing *C. diphtheria* to produce a toxin responsible for disease [28,48,49].

Immunology

Immunology is the study of how the body protects itself from disease causing microorganisms. The body has many ways to protect itself from disease by [50].

A.

1. **Mechanical mean:** Coughing, sneezing remove microorganisms from the bronchial tubes, and lungs.
2. **Cilia:** are the hair like projections) that line the bronchial tubes, and oscillate upward such that they move particles of dirt, bacteria and etc., and you cough them up.
3. **Mucus:** Produced in your nasal passages helps to trap the bacteria.
4. **Skin:** Serves as a barrier to bacteria, and etc.

Cellular: Special cells in the body protect against microorganisms. Within the lungs, special white blood cells help engulf smoke, particles, microorganisms and other debris. These Special white blood cells are actually called "alveolar macrophages," and are found within lung tissue [37].

These Macrophages actually surround and engulf bacteria, and much like amoeba projections or pseudopodia that surround, and ingest the foreign material and microorganisms. The macrophages in the lungs are stationary, there also wandering macrophages that circulate throughout the body by way of the blood and lymphatic system, to guard against invaders that try to enter cells and tissue [37].

http://classes.midlandstech.com/carterp/Courses/bio225/chap10/lecture3.htm

Serology, Agglutination and Precipitation reactions

Another important way the body protects itself is by Humoral immunity (Immunity by antibodies), and the presence of circulating antibodies within the blood serum (liquid part of the blood or plasma). There is also cellular immunity, which consists of special cells that track and remove foreign agents (bacteria, fungi and viruses) from the body [50].

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/B/Blood.html

Blood formation and Important Cell functions

These special blood proteins or antibodies are produced by plasma cells, within the liquid portion of the blood called the serum. Although at birth, we are unable for a few months to produce our own antibodies, our mother's first milk (colostrums) is rich in lactose, and many antibodies against various infections that she has overcome. As a class of proteins, antibodies are large proteins (Immunoglobulins). They are called so because they are found within serum, and particularly the "globulin fraction" (blood when been separated into its individual components) [50].

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/B/Blood.html

Bacterial Morphologies

1. Diplobacilli are two rods together or in pairs [7,30].
2. Streptobacilli Rods in chains - ex. *Streptobacillus moniliformis* cause of "Rat bite fever" [7].
3. Palisade: Several microorganisms line up together like match sticks, arrangement due to snapping type of binary fission [7,51].

For example: the coryneform bacterium are gram positive rods shaped and line up like match sticks [7,51].

Listeria is another example of an organism that palisades or line up like match sticks [7,17,51,52].

Listeria species can cause Abortion in cattle. Rods vary in size (small to large). *Listeria monocytogenes* causes "neonatal meningitis," septicemia, and stillbirths in humans. The organism causes cell division of monocytes intracellularly [7,17,51-53].

True Bacteria are cocci, rods, and spiral shaped. Coccal forms are usually about 1 micrometer size.

Spiral forms Spirochaetes (vary in size). Characteristics The cell wall is not so rigid, and is More flexible because of its endoflagella [6,7,52]. Its coils are measured by the height of one wave to the height of the next wave. Many are pathogenic and some more tightly coiled.

Leptospira is characterized by its "question mark shape". Having hooks at one or both ends. It is transmitted through water and rodents [54].

Borrelia is a cause of relapsing fever and Lyme disease. Lyme disease is caused by *Borrelia burgdorferi,* which was first reported in Lyme, Connecticut [54,55].

Treptonema: *Treptonema pallidum* is the cause of syphilis and may be detected by dark field microscopy, serological tests such as the VDRL test, ELISA and the fluorescent antibody technique [54].

Spirillum: *Spirillum volutans* is a spiral microorganism with a "rigid cell Wall," with a more exact shape [7,52].

Vibrio: the genus belongs to the family Vibrionaceae and is spiral shaped generally less than one turn characteristically looks like a "Comma"; and also has a rigid cell wall [7,52,56].

The Term Pleomorphic is defined as more than one shape during its Life Cycle [7].

Arthrobacter can change morphology when inoculated into culture media; 24-48 hours reverts from coccal to rod form, and the organism may be involved Nitrogen fixation [7].

Azotobacter is pleomorphic and is also involved in "Nitrogen fixation" [6,7,52].

Caulobacter is a stalked bacterium, but considered not a true bacteria, and may become stalked during life cycle [7,52].

Chlamydia is a "obligate intracellular parasites" and is a cause of conjunctivitis which can lead to Trachoma [7,52,57].

Chlamydia has a two cell type reproductive cycle, in which the elementary bodies are infective. The elementary bodies are attached to cells and are phagocytised. However, they are not fused by cell lysosomes. Over time they can convert to reticulate bodies that are able to undergo cell division. The reticulate bodies next undergo a change structurally, leading to the formation of glycogen staining (iodine) elementary bodies that again are infective for cells [5,7,52,57].

https://microbewiki.kenyon.edu/index.php/Chlamydia#Cell_Structure_and_Metabolism

Chlamydia species

http://plantphys.info/organismal/lechtml/bacteria.shtml

What are Bacteria & Bacterial Kingdom

http://www.textbookofbacteriology.net/structure.html

Bacterial Structure, morphology & Genetics

Structures of Bacteria

Accessory structures

Flagellum: are "whip like structures" used in motility, It helps bacteria to swim thru a liquid Medium (3,33) [6,30].

Motility Type: Not all move by Flagella.

Ex. Beggiaioa & Myxobacter: Exhibit a gliding type motility. They produce slime to glide on a surface characteristically like a slug [7,52].

Motility: Unique type, Spirochaetes can spiral thru water. Their motility due to their axial filament [6,30].

Axial filament: Probably evolution from flagellum like rope wrapped around the cell. Motility by flexing and relaxing the axial filament [6,30].

Motility: True bacteria are motile by flagella. Flagella are not visible by the light microscope alone. When tannic acid serves as a mordant, and the stain is added the size of the flagellum is coated, and its size is enlarged enough to be visible by the light microscope [2,6].

Motility: May be detected by inoculating a motility agar, and if the microorganisms is motile, it will move away from the site of inoculation [7].

Proteus: Some swarm by growing out in waves across to solid culture media [7,30].

Proteus species: Its known for its ability to swarm across across enriched media, which seems to induce the tremendous synthesis of the flagella, and the microorganism moves out as a wave of growth. This Swarming proves Proteus is motile [7,30].

The Flagellum: Originates from inside the cell thru the Cytoplasm, and extends thru the plasma membrane, and through the cell wall, and out into the surrounding medium, and originates from a basal granule or blepharoplast [6,7,30].

Blepharoplast: is the site where the flagellum is synthesized. Flagella are easily broken off, but maybe regrown and regenerated [6,7,30].

Flagella Arrangement: polar flagella occur at only one end.

Family Pseudomonadeae: are characterized by the presence of polar Flagella [7,30].

Enterobacteriaceae: usually form peritrichous flagella, which occur around the cell [7,30].

Polar distribution

Monotrichous: a microorganism with a single polar flagellum [30].

Peritrichous: Flagella are formed around entire surface of bacterium [7].

Amphitrichous: Occurs at both ends [30].

Pili = Fimbriae: are shorter, thinner and straighter than the flagellum. It is about 3-8 nanometers wide.

Pili: are chemically protein, with different serological types based on their antigenic differences [6].

Function: Some pili function for adherence, and is antigenic when inoculated into a animal, and produce known antiserum, and will react only with that specific pilus [6,7].

Type F: is called the fertility pili. It is hollow and acts as a conjugation tube. A hollow tube to which DNA passes thru to a donor cell. Those that don't conjugate lack Type F pilli [58].

Type conjugation: Gram negative rods of the family Enterobacteriaceae [30].

Synthesis of Pili: Depends on the environment from which there growing [6,7,58].

http://www.textbookofbacteriology.net/structure.html

Structure of Bacteria

http://plantphys.info/organismal/lechtml/bacteria.shtml

Cell Structure & microscopic examples of bacterial genera

Capsule, endospore type of nutritional needs

Laboratory 6 Gram negative bacteria

http://medteach.mccs.uky.edu/COM/iid98/manual/02LAB6.htm

Family Bacilliaceae: They are gram positive rods. Most are motile by peritrichous flagella. Most are widely distributed in foods. Bacillus are mostly rod shaped, and able to slit hydrogen peroxide with the enzyme catalase. They are said to be catalase positive [61].

Aerobic: an organism that grows in the presence of Oxygen for ex. Pseudomonas species [7].

Facultative anaerobic: grows in the presence or absence of oxygen, for example, Shigella sonnei [62].

Clostridium: Obligate anaerobe, sensitive to presence of oxygen (O_2), for example, *Clostridium perfringens,* which is a cause of "gas gangrene" [7,49].

Sporosarcina: is an endospore forming coccus that occurs in tetrads. It forms spore/ bacterium, which is not reproductive mechanism. The mechanism is for adverse conditions [7].

Resistance: is high, and requires 121 °C for 15 minutes of endospores to kill the endospore.

Autoclave: kills microorganisms, bactericidal and sporocidal at 121 °C for 15 minutes [7].

http://www.google.com/search?sourceid=navclient&ie=UTF-8&rlz=1T4GGLT_enUS393US393&q=Bacterial+Structure+%26+Function

http://www.shmoop.com/biology-cells/prokaryotic-cells.html

Sporogenesis

During the Stages of Sporogenesis there is an invagination of the cytoplasmic membrane, in the area where the spore is going to form. Spores are forming in different places depending on the genetic material of the cell [12,52]. Endospores may sometimes form centrally, subterminally, terminally. Knowing where the endospore is developing the "Sporangium" can give a clue of what the organism might be. It is necessary to know if the sporangium is swollen? Does it swell, the location of the endospore is of taxonomic value. The genus Bacillus is placed into five groups based the position of the endospore, as well as whether the sporangium is swollen [12,52,61,63].

Uses of Bacterial Polysaccharide

Capsule: may be polysaccharide, polypeptide; or cellulose depending on the genera [6,30].

Function: Capsule may contribute to the virulence of a microorganism [7].

Cellulose: Acetobacter produces a capsule material [7]. Material Immunologically distinct if one injects a capsule into an animal, and is antigenically specific, it can elicit an antibody response [49].

Quellung Reaction: Quellung reaction is a reaction which distinguishes the capsular type that microorganisms exhibit by the swelling of the capsule [7].

Streptococcus pneumonia; has capsular types that can be identified serologically during "pneumonia," and can be used to distinguish an unknown "capsular antigen" with known antiserum. If swelling occurs, it is a positive reaction "Quellung reaction," [6,7].

http://textbookofbacteriology.net/structure_4.html

Provides Classification - structure & morphology

http://www.atsu.edu/faculty/chamberlain/website/lects/bacteria.htm

Cell Wall structure (molecular), Capsule & flagella

http://www2.estrellamountain.edu/faculty/farabee/BIOBK/BioBookDiversity_2.html

http://biology.clc.uc.edu/courses/bio106/bacteria.htm

Just very basic structure & morphology (Diplo, Strepto)

In the quelling reaction the microorganisms appears as though there is a swelling, it isn't really occurring "Quellung reaction" [7]. Prior to the advent of antibiotic therapy, antisera was used to treat capsular bacterial infections, for example, in some circumstances *Haemophilus influenza* infections were treated by the use of antisera. Today these infections are treated by antibiotics [7,49].

Colony: A Capsule may be waxy in appearance, and or mucoid in appearance and or shiny in appearance [7,49].

Capsule: A capsule may be waxy in appearance, and or mucoid in appearance, and or shiny in appearance, in culture (7,49) can form a slime on food materials. An extract of which can be used as a plasma extender by mixing plasma with dextran [6,7]. The problem in the Food Industry the microorganism can form slime on the food products, for example: *Leuconostoc mesenteroides,* and *Haemophilus influenza* [6,7,64].

Dextran can be extracted and can used as a plasma extender [7].

Polymerized can use as a plasma extender by mixing plasma with dextran [7].

Dextran Sucrose: When an organism is supplied with sucrose, it only polymerizes the glucose component, and Polymerize selects for only the sucrose in the media. When polymerizing the fructose moiety and making a Levan molecule, it requires the total fructose molecule. It can not polymerize glucose itself, it must extract glucose from the sucrose molecule. This is because the organism produces dextranase which sucrose as its substrate [7,65,66].

The organism Acetobacter also forms a capsule, which consists of cellulose, whereas *Bacillus anthracis* forms a polymer of D-glutamic acid [7,52]. The ability to form a capsule may contribute an organism's ability to cause disease. This is because the ability to form a capsule interferes with the normal process of the immune system namely phagocytosis. The presence of a capsule enables the microorganisms to continue to establish a infection, and virulent. Virulence is therefore the ability to produce disease, in some cases the presence of a capsule enables the organism to be disease causing or virulent [7,67].

Streptococci some species may also forms a capsule. The presence of a capsule may contributes to its virulence, it can cause "Strept throat" and may produces a capsule made of "hyaluronic acid" [7,65].

Capsule types: Some form capsules that are glycoprotein (proteins bonded to carbohydrates) or glycolipids ("carbohydrates bonded to lipids") [30,48,66].

Capsules In the past antiserum was derived from capsular antigens, and used to treat an infection. When the antisera comes in contact with the capsule, it appears to swell, when viewed microscopically. The microorganism appears as though its swelling, but it doesn't really occur. It just appears to swell when looking under the microscope. We now use antibiotics instead of antisera to treat difficult capsulated bacteria [7]. Capsule's may appear waxy in appearance and some mucoid in appearance, and may cause a slimy appearance on food [7]. *Streptococcus pneumonia* is a cause of many diseases such as pneumonia, bacteremia, and endocarditis, and it also forms a capsule that can prevent phagocytosis [7,68].

Microorganisms have a number of ways in order to overcome the immune system, and not just by forming capsules. Microorganisms also have what is called virulence factors. Virulence factors are factors that act like enzymes, that can cause an elevation of one temperature, cause damage to either blood vessels or blood cells, tissue in general [7,69].

http://textbookofbacteriology.net/themicrobialworld/homepage.html

http://textbookofbacteriology.net/bacteriology.html

Various Areas that Prokaryotes are located in the World

Nutritional Needs

1. Microorganisms need a carbon source for *energy.*
2. They need a nitrogen source for protein synthesis and nucleic Acids [70].
3. Also a salt source to maintain osmotic pressure [11,12].
4. They Require minerals that serve as cofactors, and or allow an enzyme to that fit its substrate. For good growth, they need a balance of Osmotic Pressure and proper pH, Some bacteria require certain minerals to make in proper molecular configuration of functional proteins [11,12].
5. Phosphates ($P0_4^{-3}$) are important in the Energy Transferring system (ATP synthesis) [7,11].
6. Coenymes have ($P0_4$) in their molecule, for example: Nicotinamide adenine dinucleotide (NAD, NADP) Flavin Adenine (dinucleotide Flavins), and the Electron Transport System. It is an enzyme that serves as an electron carrier in the electron transport system [12,38].

   Phosphates ($P0_4$) occur in (Nucleic Acids) and are required in phospholipids which occur in plasma membranes. They are added in the form of KH_2P04, K_2HP0_4 as buffers to culture media [7,11,12,71,72].
7. Potassium ion (K+) is involved in a variety of enzymes, protein synthesis and ion transport system [7,70,71].
8. Calcium The calcium (Ca++) ion is involved in maintaining the structure of the cell wall [7,30]. The Calcium ion is also a component of dipicolinic acid a chemical in endospores [30]. Endospores are necessary because they help the organism to survive under adverse conditions [7,30,70].
9. Cell Wall The internal components of the cell wall give cells the attribute of being either Gram+ or Gram-. The chemical differences of cell wall of other microorganisms is of taxonomic importance [7,30].

Its major structure is composed of a layer of alternating units of N-acetyl-glucosamine and N-acetyl-muramic acid, the dissacharides which make the backbone of the cell wall, and are linked together by β-1-4 Linkages. Each muramic acid layer is lined by a trail of four polypeptides called the tetra-peptide. The tetra-peptide consists of L-Alanine, D-Glutamic acid, and D-Alanine and lysine for *Staphylococcus aureus*. D,L-Diaminopimelic acid (meso-DAP) is substituted for lysine in *Escherichia coli*. The tetrapeptide extends from the lactyl group on C3 of the Muramic acid, and cross links to the adjacent muramic acid strand. In the case of *Staphylococcus aureus* it is cross linked by 5 glycine molecules, whereas *Escherichia coli* is cross linked by the tetrapeptide with the exception that meso-DAP is substituted for lysine in the tetrapeptide of gram negative bacteria [7,30,48].

http://www.atsu.edu/faculty/chamberlain/website/lects/bacteria.htm

Cell Wall dissacharides, periplasmic space & Cross bridge

http://www.jlindquist.net/generalmicro/102bactnut.html

Nutrition & Cultivation of Bacteria

Cell Wall Continues

The Cell Wall is responsible for giving the bacterium its shape, size, morphology and stainability. Its Serological behavior, and its certain antigenicity, and elicits antibody formation [6,30,44,73].

Cell wall if extracted, it stimulates antibody formation, and necessary for phage absorption and motility. It contains many antigens, and has an affinity for certain specific stains [6,30,74].

Cell Wall's Structure differs between Gram positive and Gram negative microorganisms. It ranges in depth between 20-80 nanometers (0.020 micrometers) and is composed of proteins and polysaccharides. Penicillin inhibits synthesis of the cell wall of Gram positive bacteria, however, doesn't always kill the bacterium. It just limits the cell wall from forming the cross bridge between adjacent strands from being put together for ex. *Staphylococcus aureus* [28]. However, some microorganisms lack a cell wall. Both Mycooplasma and Ureoplasm lack a cell wall. They are pleomorphic, and able to exist in more than one morphology [28,30].

Periplasmic space: in gram positive bacteria and archaea occurs between plasma membrane and cell wall, whereas in gram negative bacteria, it lies between the cytoplasmic membrane, and the outer membrane. The periplasmic space contains many antigens. It is also a site of porins and transport [6,7].

Teichoic Acids: Teichoic acids are also associated with the cell wall, and are probably synthesized with nucleoside sugars, and then

transmitted to the cell wall, with gram positive microorganisms. Again Gram positive microorganisms have teichoic acids (TA) that are associated with the cell wall. Some Teichoic acids may also be associated with the cell membrane. Teichoic Acids are characterized as repeating units of either glycerol or ribitol connected by phosphate ester bonds [7].

Absence of Cell Wall

If a microorganism is devoid of their cell wall, it may be referred as a spheroplast. An organism is referred as a spheroplast, if it still has cell wall fragments attached to the cell wall (remnants) [30]. It is called a Spheroplast if the cell wall (c.w.) is left only with fragments still (remnant of C.W.) attached it is called a spheroplast [30].

To form a protoplast it involves a chemical reaction of the cell. It involves some type preparation or digestion of the cell wall. For example, the method to form a protoplast is by digestion of the cell wall by enzymes or detergents [30].

L-forms are microorganisms that exists naturally without a cell wall, both mycoplasm and ureaplasm are devoid of a cell wall [30]. The Genus Mycoplasma and Ureaplasma when grown on culture media, it has its characteristic "Fried egg appearance [5,30].

Inclusions

Metachromatic Granules

These granules appear as relatively large granules that may stain red with blue dyes, and particularly methylene blue dye. They are often referred to as "Volutin", which may serve as a food reserve, or as a source of phosphate for the synthesis of ATP [7,30].

Cytoplasmic Inclusions may be:

1. Polysaccharide
2. Lipid
3. Sulfur
4. Carboxysomes
5. Gas vacuoles
6. Magnetosomes [30].

Inclusions

Cytoplasmic granules may be polysaccharide, in the form of starch or glycogen and made visible by the application of iodine, (glycogen reddish brown), whereas starch granules appear blue [30]. Cytoplasmic are inclusions granules that represent accumulations of food reserves which can occur as polysaccharides, lipids, or polyphosphates. Starch is the storage product of *Neisseria* and *Clostridium* species [7,28].

Both the genus *Pseudomonas* and *Bacillus* use glycogen as a storage product, and may be observed microscopically when stained with sudan black. The lipid material in the form of poly-beta-hydroxybutyrate, is stored by *pseudomonas* and the genus bacillus, whereas metachromatic granules (volution) are a food reserve for Corynebacteria, Yersinia pestis and mycobacteria. The metachromatic granules when stained with methylene blue appear reddish pink, and can be seen under immersion oil under the microscope [7,28].

http://classes.midlandstech.edu/carterp/Courses/bio225/chap11/index.htm

Bacterial List of genera description, Oxygen requirement, chemolithotroph or heterotroph & temperature for growth

http://www.textbookofbacteriology.net/structure.html

Bacterial Structure

http://www2.estrellamountain.edu/faculty/farabee/BIOBK/BioBookDiversity_2.html

Bacterial Structure- Cell Wall, Peptidoglycan, Periplastic space

Bacterial- diversity of Groups

Nutrition

Microorganisms require various nutrients in order to grow and reproduce and assimilate cell protoplasm [11,12].

Nutrition is a twofold process:

A. They synthesize protoplasm from this nutrition

B. Nutrition supplies "energy" for the cell so it can carry on out reactions, making possible synthesis of substances necessary for cell growth [7,11,12].

Culture media is the way we supply nutrients to microorganisms in a variety of forms, i.e. classify on the basis of the type nutrients utilized, and of taxonomical value [7,11,12].

http://www.jlindquist.net/generalmicro/102bactnut.html

Forms of different types of respiration

Metabolism

1. Is the sum total of all reactions that occur within an organism.
   a. Anabolic reactions require energy to synthesize more complex compounds.
   b. Catabolic reactions are reactions that release energy by the breakdown of chemical bonds.

   Microorganisms actually obtain energy by oxidation and reduction. Oxidation is the loss or removal of electrons, whereas reduction is the gain of electrons. Therefore, when chemical bonds are broken, energy is released and can be captured, and used for the synthesis of adenosine triphosphate (ATP), the energy currency of the cell [38].

Microorganisms need to perform many of these functions in order to obtain a source of carbon to synthesize carbohydrates, proteins and fats [38].

2. Mechanisms of carbon acquisition by organisms [38]. Microorganisms are able to obtain energy and acquire the carbon they need through the following means:

a. Autotrophic- an organism acquires its energy from sunlight an carbon from carbon dioxide "CO_2" [38].

b. Heterotrophs- an organism that obtains its carbon from preformed forms of carbon, or organic compounds [38].

c. Photoautotrophs obtain energy from photosynthesis, and use CO_2 as their source of carbon [38].

d. Chemoautotrophs use other inorganic compounds (such as sulfides and nitrites) as their source of energy, and carbon dioxide as their carbon source [38].

e. Photoheterotrophs are heterotrophs that obtain their energy from light. A small number of bacteria use energy from light, but require organic substances (alcohols, fatty acids and carbohydrates) and so forth [38].

f. Chemoheterotrophs, obtain their carbon and energy source from the break-down of ready made "organic compounds," fungi for example [38].

g. Saprophytes get their carbon from decaying organic matter [7,38,39].

h. Parasites must obtain their carbon from other living organisms [39].

http://textbookofbacteriology.net/themicrobialworld/metabolism.html

http://www2.estrellamountain.edu/faculty/farabee/BIOBK/BioBookDiversity_2.html

Substrate level phosphorylation-and ATP formation

Metabolic Methods for acquiring Energy

2 Major Categories

1. Phototrophs use light as an energy source and CO_2 as a carbon source [39].
2. Chemotrophs depend on oxidation-reduction reactions of inorganic or organic compounds for their primary source of carbon [7,39].
3. Phototrophs capture radiant energy of sunlight, and transform it into chemical energy that is stored in the bonds of carbohydrates, and other molecules [7,39]. Phototrophs include Green plants, Algae, Cyanobacteria and Photosynthetic bacteria [39].

http://textbookofbacteriology.net/themicrobialworld/metabolism.html

http://www2.estrellamountain.edu/faculty/farabee/BIOBK/BioBookDiversity_2.html

Most organisms are unable to use radiant energy of the sun and must instead rely on the oxidation of chemical compounds as a source of energy and are called chemotrophs [7].

Chemoorganotrophs and Chemolithotrophs

Chemoorganotrophs however, obtain their energy from the oxidation of organic compounds [7,39]. Most bacteria are chemorganotrophic and use organic compounds such as carbohydrates, organic acids, and proteins for their energy source [7,39].

Chemolithotrophs obtain their energy from the oxidation of inorganic compounds such as hydrogen sulfide (H_2S), hydrogen gas (H_2), nitrite ($NO\text{-}_2$), ammonia (NH_3) and ferrous iron (Fe^{+2}) [39,52].

Winogradsky showed that Beggiatoa, a filamentous gliding bacterium found primarily in sulfide-rich habitats, can oxidize H_2S in these environments, first to elemental sulfur (So), and then to sulfate (SO_4^{2-}) [7,19,52,75].

http://www2.estrellamountain.edu/faculty/farabee/BIOBK/BioBookDiversity_2.html

http://www.cas.miamioh.edu/~stevenjr/mbi202/bacteria202.html

List of bacterial genera & metabolic processes

http://www.russia-ic.com/people/education_science/w/311/

Winogradsky biography on his life

Carbon is required by living organisms as an important constituent of cellular structure and metabolic compounds. This element exists in many forms in the environment. Carbon may appear in a simple form as gaseous carbon dioxide, or as more complex organic compounds. Microorganisms are remarkably diverse in their carbon requirement and are divided into two groups the Autotrophs and Heterotrophs based on their source of carbon [11].

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

Metabolic process of Autototrophic & Heterotrophic bacteria

http://textbookofbacteriology.net/metabolism.html

http://textbookofbacteriology.net/environment_2.html

Autotrophic microorganisms synthesize organic substances from carbon dioxide through a process known as carbon dioxide fixation by the Calvin Cycle [39]. The carbon cycle provides 3 and 5 carbon sugars that are utilized to assimilate into amino acids, fats and purines and pyridines which make up DNA and RNA [7,39]. Autotrophs are important in nature because through the carbon dioxide cycle, they provide the organic substrates that form the basis of the food chain for other organisms [38,39]. Autotrophic bacteria include those bacteria that obtain their energy from light (Photoautotrophs), and those that obtain their energy from inorganic compounds are "chemoautotrophs," [38,39].

Heterotrophic bacteria use organic compounds as their carbon source. Microorganisms unable to use carbon dioxide as their principle source of carbon, instead, require preformed organic compounds for their carbon. These microorganisms are called heterotrophs [38,39].

Chemoheterotrophs are organism's that oxidize chemical compounds for their energy and require organic forms of carbon [38,39]. Heterotrophic bacteria vary considerably in their requirements for organic substrates [38,39]. Others like the

methane oxidizing bacteria of the family Methylomonadaceae, use methane and a few other 1 - carbon compounds as their sole source of energy and carbon [7].

http://textbookofbacteriology.net/metabolism.html

http://textbookofbacteriology.net/procaryotes_4.html

http://textbookofbacteriology.net/environment_2.html

Oxygen requirements

http://textbookofbacteriology.net/nutgro.html

http://textbookofbacteriology.net/environment_2.html

http://textbookofbacteriology.net/metabolism_6.html

Other Distinctions

Although photosynthetic bacteria are photoautotrophic in the presence of light, some of these same microbes may function Chemoautotrophically in the darkness, using compounds such as hydrogen sulfide (H_2S), thiosulfate ($S_2O_3^{2-}$), and molecular hydrogen (H_2) as energy sources [11,38,39]. Certain photosynthetic bacteria can use organic compounds as carbon sources, and are thus called photoheterotrophic [12,39].

Oxygen requirements

Various types

1. Aerobic - bacteria grow in the presence of oxygen [38,39].
2. Microaerophilic- bacteria grow best at oxygen concentrations lower than that of atmospheric air [38,39].
3. Facultatively Anaerobic - bacteria that grow in the presence or absence of oxygen (they respire in the presence of oxygen and ferment in the absence of oxygen) [38,39].
4. Anaerobic - bacteria grow best in the absence of oxygen [12,39].
5. Obligate Anaerobes - are microbes that will not grow even in a trace presence of oxygen "too toxic," [11,12,38,39].
6. Aerotolerant Anaerobe - they survive and grow in the presence of small amounts of oxygen, although they do not use oxygen metabolically [7,12].

http://textbookofbacteriology.net/environment.html

http://www.cas.miamioh.edu/~stevenjr/mbi202/bacteria202.html

List of Genera metabolism & Oxygen requirements

http://textbookofbacteriology.net/metabolism_6.html

Diversity in Metabolism in Procaryotes

Concepts of Metabolism

Most microorganisms obtain their energy by the oxidation of chemical compounds. This breakdown of chemical compounds is called catabolism. Anabolism is the processes associated with the biosynthesis of chemical compounds, whereas metabolism is the sum total of the reactions (catabolic & anabolic) carried out by the cell [38,39].

Metabolism

Fermentation and Respiration

Basic Processes: fermentation & respiration

In fermentation, an organic substrate serves as the electron donor; an oxidized intermediate of the substrate acts as the final electron acceptor, and subsequently is reduced. Because fermentation does not require the presence of oxygen, microbes that ferment carbohydrates may do so in the absence of oxygen [38,39].

http://www.cas.miamioh.edu/~stevenjr/mbi202/bacteria202.html

Biodiversity of Microbes, modes of nutrition & metabolism

http://textbookofbacteriology.net/metabolism.html

Microbial Metabolism

Respiration

Respiration on the other hand, requires an external electron acceptor for substrate oxidation. When molecular oxygen is the terminal electron acceptor, and is reduced to H_2O; this process is called aerobic respiration [39]. Another inorganic molecule such as nitrate (NO_{3-}) or sulfate (SO_4^{-2}) may serve as the terminal external electron acceptor (Anaerobic respiration) and becomes reduced to nitrite (NO_2-), nitrous oxide (N_2O) and nitrogen (N_2), or to hydrogen sulfide (H_2S) and is anaerobic respiration [38,39].

Although respiration differs in their mechanisms for electron transfer, both fermentation and respiration use substrate oxidation to channel energy from chemical compounds into energy-rich molecules such as Adenosine Triphosphate (ATP) [38,39].

http://www.cas.miamioh.edu/~stevenjr/mbi202/bacteria202.html

202 General Microbiology II "Metabolic Respiration"

http://textbookofbacteriology.net/metabolism.html

http://www.cas.miamioh.edu/~stevenjr/mbi202/bacteria202.html

Nutrition

2 Types of Nutrition

1) Autotrophic organisms use sunlight for energy and CO_2 as their carbon source.

2) Heterotrophic use organic compounds as their carbon source

a) Saprophytic use nonliving organic materials for the nutrition, whereas,

b) Parasitic use living organic materials [39].

"**Nutrition Requirements**" whether organic & or others.

http://textbookofbacteriology.net/metabolism.html

http://textbookofbacteriology.net/nutgro_3.html

www.jlindquist.net/generalmicro

Catabolism, Metabolism and Fermentation

1.Nutrients such as C, N, Minerals (Fe, mg, mn, Ca), Salts are considered as trace element requirements [12,70].

2. Moisture - 75% - 85% in bacteria or spores [11,12,70].

3. pH ------ proper 6-8 [11,12,76].

4. Temperature 37 °C, 25-40 °C -Mesophilic [11,12]. Up to 42 °C Campylobacter "Thermophiles" Endospores are formed by *Clostridium & Bacillus* (mesophiles) [28,77].

Cryophilic ----- below 20 °C [12,78].

Psychrotrophic - (O °C - 5 °C)

5. Light --------- Ultraviolet light-UV "surface disinfection," [12].

6. Oxygen (O_2)---- requirement for some microorganisms [12].

Other oxygen requirements

First microbial life is believed to be anaerobic.

1. Anaerobic bacteria - grow in the absence of oxygen [12].

E.g. *Bacteroides fragilis* (causes abdominal wounds)

2. Aerobic - grow only in the presence of oxygen, e.g. *pseudomonas* and *micrococcus*. These microorganisms can reduce the oxygen concentration for anaerobes to grow [12].

3. Facultative organisms grow either in the presence or Anaerobic absence of air [12]. Most pathogens are Facultative Anaerobes [12].

4. Microaerophilic *N. gonorrhoae* and grow in minute amounts of oxygen. Campylobacter and also Viridans streptococci [7,12,79].

5. Capnophilic (Capneic): like increased carbon dioxide (CO_2) 5-10%. The anaerobic breakdown of carbohydrates and related fermented compounds, in which products formed serve as final electron acceptor for hydrogen [7,12].

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

http://www.cas.miamioh.edu/~stevenjr/mbi202/bacteria202.html

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

http://www.bing.com/images/search?q=Campylobacter+Agar&FORM=IRMHIP

Campylobacter on a variety of different culture media

Growth parameters

Fermenters: are organisms that produce acidity throughout culture Media [7]. Nutrients for growth, such as TSI (Triple Sugar iron Agar), LIA (lysine iron agar), or any media with a good carbohydrate source [80,81,82].

E.g. *Escherichia coli*, *Salmonella typhi* and *Enterobacter* [7].

Nonfermenters: are organisms that produce weak levels of acidity in culture media [80,81,83]. They are often grown in a media like Hugh Leifsons OF Medium, since it contains low levels (0.2%) of peptone [7,80,81].

http://www.powershow.com/view/108556-Yjk5O/NonFermentative_GramNegative_Bacteria_powerpoint_ppt_presentation

A Non-fermenter is a microorganism that is a gram negative bacillus which are unable to use carbohydrates either as a carbon source, or energy source, and which has special nutritional requirements [82]. Other - media which contain 1% peptone, these microorganisms are called nonoxidizers, since they produce alkaline end products in large volumes [81].

These Nonoxidizers produce alkaline products which may overwhelm the small volumes of acid produced by these microorganisms, and thus cause the pH indicator to change to a alkaline color [7,73,81].

Glucose Nonoxidizers would include the genera Moraxella, Alcaligenes and A*cinetobacter calcoaceticus* variety anitratus (renamed as *Acinetobacter baumanni)* or *Acinetobacter calcoaceticus* lwoffe [73,81,84].

http://www.bing.com/images/

Examples of nonoxiders are *Acinetobacter, Moraxella catarrhalis*

http://delrio.dcccd.edu/jreynolds/microbiology/2420/BIOL%202420%20lab%20manual%20TOC.html

List of Biochemical tests for bacteria

http://www.google.com/search?hl=en&rls=RNWE%2CRNWE%3A2005-02%2CRNWE%3Aen&q=Classification+and+identification+of+Nonfermentative+Bacteria&btnG=Search

http://faculty.ksu.edu.sa/manalk/Documents/%d9%86%d8%b3%d8%ae%20%d9%85%d9%86%20Non-Fermentative%20Gram-Negative%20Rods.pdf

Nonfermentative bacteria- biochemical tests

Excellent and Pseudomonas growth on Agar and Vibrio Species*

http://www.powershow.com/view/108556-Yjk5O/NonFermentative_GramNegative_Bacteria_powerpoint_ppt_presentation

http://delrio.dcccd.edu/jreynolds/microbiology/2420/BIOL%202420%20lab%20manual%20TOC.html Broad list of culture methods biochemical tests

Oxidizers are microorganisms that grow and convert glucose to acid at the surface of OF Medium and do not change the indicator in the sealed medium e.g. *Pseudomonas aeruginosa* [73].

Pseudomonas aeruginosa and *Acinetobacter anitratus* are examples of oxidative organisms. Also Flavobacteria are glucose oxidizing organisms that do not produce gas from glucose[7]. oxidizing organisms do not produce gas from glucose [42].

With nitrate reduction most bacteria reduce NO_3 to NO_2 [7].

P. aeruginosa and a few other species (All Enteric's) are able to reduce Nitrates (NO_3) to nitrites (NO_2). *P. aeruginosa* reduces nitrate to nitrogen gas [7,86].

Denitrifiers *P. denitrificans* is a strict aerobe but capable of "Anaerobic respiration" with nitrate as the final electron acceptor (under anaerobic conditions) [7,70].

Nitrate- (NO_3) rather than free molecular oxygen, becoming the terminal acceptor of hydrogen derived from oxidation of a energy source [7].

http://www2.estrellamountain.edu/faculty/farabee/BIOBK/BioBookDiversity_2.html

Denitrifiers

http://www.bing.com/images/

Acinetobacter species

Biochemical tests & Culture Media

http://www.slideshare.net/doctorrao/nonfermenting-gram-negative-bacteriapptx12

http://faculty.ksu.edu.sa/manalk/Documents/%d9%86%d8%b3%d8%ae%20%d9%85%d9%86%20Non-Fermentative%20Gram-Negative%20Rods.pdf

Pseudomonas

http://www.google.com/url?

Culture Methods

With the development of agar as a solidifying agent; Cultivation of microorganisms at 35 °C became possible, as well as the ability to study isolated microorganisms, or microorganisms in pure culture [12].

What is a Pure Culture?

A pure culture is a culture that has arisen from one colony type, or species of microorganism. These colonies actually represent a population of microbial cells; growing in, or on solid culture media; and arising or originating from a single microbial cell [7,12]. During a colony is a macroscopic "Visible growth" of a microorganism on a solid culture medium. Culture Media is really for the growing of microorganisms [7,12,86].

History of the Culture Media Technique

In 1880 the use of potato's, eggs, and or some other naturally occurring foods, were the primary means for culturing microorganisms. In the laboratory a microbiologist often used gelatin as their medium for culturing microbes. However, with gelatin it remains a liquid at 35 °C or 95 °F [12,87]. Therefore gelatin is not suitable for growing pathogens, since it will not form a solid surface for culturing pathogens at the normal temperatures 35 °C (95 °F) that pathogens are cultured [7,12,88].

Therefore, during this particular period of history, it was virtually impossible to grow pathogenic microorganisms. Often because the materials that were available for growing the microorganisms were generally not feasible for cultivating the pathogenic microbes, which required a growth temperature of 95 °F [7,12,88].

However, it wasn't until the Hess's wife suggested that agar be substituted for gelatin as a solidifying agent, that it became possible for pathogenic microorganisms to be grown as pure culture in sterile Petri dishes. Microbiology owes a great deal to W. Hesse's Wife, one of Dr Koch's assistants, Dr. Hess's wife noted that broth's with agar added formed to a solid surface in petri-dishes, and could be grown at 35 °C the same temperature (body temperature) that pathogens need for growth [7,12,88].

Hess and his Wife knew of agar since they used it originally as a solidifing agent in jams and jelly's. Agar is formed from the red alga Gelidium, and has the unique property of being a liquid at boiling temperatures. But also, it can be cooled or tempered to just above 46 °C, and remain a liquid to be poured into petri dishes, or other vessels and will solidify when cool [7,12,88].

The advantage, therefore or quality is that as the temperature of the culture drops below 45 °C, agar based media gels or solidifies. Thus providing a solid surface for cultivation of pathogenic microorganisms, since it remains a solid at 35 °C. The normal temperature upon which most microorganisms, as well as pathogenic microorganisms grow [7,12,88].

http://users.stlcc.edu/kkiser/history.page.html

History of Microbiology

Identification Methods

1. Direct Examination: to observe the morphology of microorganisms, their size, shape, and structures by using staining methods.
2. Cultural Characteristics: what the microbe looks like on Culture media [12].
3. Biochemical Characteristics: The type of substrates a microorganism is capable of utilizing for growth. For example. The Oxidase test forms a purple color due to the presence of the enzyme Cytochrome oxidase [2,7,35].

http://rcpa.tv/parts/educational/microbiology/marion_yuen.pdf

Gram Stain, Various genera Strept., Moraxella, Coccobacillary

Rods, Streptococci Acinetobacter On Blood Agar

Differential stain: 1. Gram stain and 2. Acidfast stain

Dyes and stains (Biological)

Stains are dyes that are used in microbiological staining. They are generally aniline, that is they are chemically basic, and because of the "acidic properties (anionic) or nature of the cell wall, can explain their ability to attach or chelate, and thus stain the Cell Wall. Acidic stains include eosin, acid fuchsin, picric and nigrosin. These stains are repelled by the slight anionic nature of the cell wall, and so only coat the cell. Basic dyes like crystal violet, methylene blue, and malachite green are attracted

to the negatively charged cell, and thus bond to the cell or cell components [2,7,13,45,89].

Although most stains (simple or differential) in themselves are capable of staining cell components. Some dyes or stains have only a limited affinity for microbiological components. Some dyes or stains must work in conjunction with special chemicals called mordants. These substances (mordants) form a "insoluble" compound" with a stain, and help fix the color to the bacterial cell. With a mordant in conjunction with a stain, they permanently affix the color to the bacterial cell [2,7,45,89].

A simple stain is the application of a single stain to a heat fixed smear, for the purpose of staining. Stains that involve two or more stains are often referred as differential, since they can help distinguish between morphologically similar but different parts of the cell [2,7,45,89].

http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/gram.htm

Gram staining

http://www2.estrellamountain.edu/faculty/farabee/BIOBK/BioBookDiversity_2.html

Biodiversity

Acidfast staining

When staining a heat fixed slide, carbofuschin is applied first and gently heated for several minutes. It is then washed off with water and then acid alcohol is used as the decolorizer, and then again washed off. The slide is then counterstaned with methylene blue. Acidfast microorganisms appear red, whereas nonacidfast organisms when counterstained with methylene blue appear blue [2,7,45,90]

http://www.bing.com/images/h?q=capsule+and+flagella+stain&qpvt=Capsule+and+flagella+stain&qpvt=Capsule+and+flagella+stain&FORM=IGRE

Various stains- gram, capsule, flagella, & endospore below

http://delrio.dcccd.edu/jreynolds/microbiology/2421/lab_manual/TOC.html

http://www2.estrellamountain.edu/faculty/farabee/BIOBK/BioBookDiversity_2.html

Acidfast Procedure

Laboratory Preparation - Additional staining methods

I. Examination of Fixed, Stained Material Staining types

A. Simple - using one dye that serves to delineate "morphology," but renders all structures the same hue.

B. Differential consist of more than one dye added in several steps, and the stained structures are "differentiated" by color as well as shape [45].

Preparation of Smear:

1) Once a swab is touched to a slide it is nonsterile and cannot be inoculated to Sterile media (Prepare Smears & specimens) [45].

2) Emulsify specimen in a drop of H_2O "Sterile Water" is best [7,45,91].

http://delrio.dcccd.edu/jreynolds/microbiology/2421/lab_manual/TOC.html

Smear Preparation

Many times bacterial slides may be best prepared with sterile water. If the slide is not "heated", formalized, or autoclaved, organisms may "survive" the Staining procedure [45].

Gram Stain is credited to Christian Gram [7]. It Separates--Microorganisms into two Large groups. Those that take up Crystal Violet "CV" (gram positive) and those that allow C.V to wash out easily with the decolorizer (alcohol or alcohol/acetone) and are Gram Negative (Red). Fixing is generally accomplished by "flaming" or by "fixing" with methyl alcohol [7].

http://en.m.wikipedia.org/wiki/Blood_film Blood elements shown

http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/gram.htm

Gram Stain

http://www.bing.com/images/h?q=gram+stain+procedure&qpvt=gram+stain+procedure&FORM=IGRE#a

Gram Stain method

http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/gram.htm

http://www.bing.com/images/h?q=capsule+and+flagella+stain&qpvt=Capsule+and+flagella+stain&qpvt=Capsule+and+flagella+stain&FORM=IGRE

Gram stain, Capsule, Acidfast & Endospore stain, Excellent

Cultural and Visual Methods

Using microscopes make visualizing microorganisms possible. Microorganisms are objects as small as 200 nanometers or smaller, for example Pox Virus are even smaller at 200 nanometers [7].

Example below RBC- 7-8µ [92], *Escherichia coli* at 1.5 micrometers µm and *Staphylococcus aureus* 1µm [7].

Pure Culture

A Pure Culture is a culture that has arisen from one "colony type" or species of organism, whereas a colony may be defined as a "macroscopic visible growth of a microorganism" on a solid culture medium. Culture Media is the source of nutrients required for the growing of microorganisms [12,35,86].

Identification Methods

1. Direct Examination "morphological," size & shape and structure (Gram reaction) [7].
2. Cultural Characteristics what the microorganism looks like on culture media.
3. Biochemical Characteristics are the type "nutrients" its capable of italizing for growth [7,35,89]. The Oxidase test

strip turns purple due to the presence of cytochrome oxidase [7,28,90,93]. Both *Neisseria meningitidis* and *N. gonorrhoeae* are gram negative diplococci (occurs in pairs) with adjacent sides flattened [7,12,94]. Chocolate Agar, Thayer Martin Lewis and will grow the genus Neisseria) [83]. However, confirming by carbohydrate fermentation is discouraged and also [89,94].

4. Animal Inoculation is by inoculating intraperitonally "IP" [95].
5. Methods are antigen (ag) and antibody (ab) reactions and called "Serological Methods," [29,45,96,97].

http://classes.midlandstech.com/carterp/Courses/bio225/chap10/lecture3.htm

Serological and biochemical methods of identification

http://www.bing.com/videos/h?q=serological+techniques&qpvt=Serological+Techniques&FORM=VDRE

Cultural, Biochemical identification, Fluorescent Antibody Technique (FAT), Serological & DNA hybridization methods of Identification.

Direct Examination

A. Unstained Methods

1. Wet mount is used to observe the organism in its natural state, placed in a drop of water on a slide. Many times wet mounts can help to determine whether an organism is motile [7,40].
2. Staining helps to provide the morphology (shape, size).

B. Darkfield Microscopy - A dark field adaptor blocks off light, and only oblique light enters indirectly. The advantage is, one can observe microorganisms in their natural state [45]. *Treptonema palladium* is the cause of syphilis and forms and characteristic chancre. It can also be seen by darkfield microscopy [40,45].

http://www.jlindquist.net/generalmicro/index.html

http://delrio.dcccd.edu/jreynolds/microbiology/2421/lab_manual/TOC.html

Direct Microscopic Methods

http://www.austincc.edu/microbugz/handouts/Stain%20protocols.pdf

Cultural methods and selective & differential media

http://generalbacteriology.weebly.com/culture-media.html

Cultivation of microorganisms

1. Cultivation: begins with providing a culture media that supplies all the necessary nutrition.
2. Biochemical tests are often used to characterize the variety of biochemical substrates that an isolate might utilize. Biochemical tests help to provide data that may contribute to a identification of an unknown isolate [7,28,29,35,90,93].
3. Serological methods are often used as an aid in recognizing an organism, which may be further identified by biochemical tests [29,88].
4. Animal inoculation - animals may be inoculated intraperitonally," and primarily is used for research purposes [95].

http://delrio.dcccd.edu/jreynolds/microbiology/2421/lab_manual/TOC.html

Culture media and staining methods

https://images.search.yahoo.com/search/s;_=A0LEV2CEtZNTelYAZt1XNyoA;_=X3oDMTB0a3VnZmkwBHNlYwNzYwRjb2xvA2JmMQR2dGlkA1NNRTQ4NV8x?_adv_prop=image&fr=altavista&sz=all&va=bacterial+culture+media

General Purpose Media- will provide the necessary nutrients for Growth for a variety of microorganisms. However, some organisms have a more exacting nutritional requirement [7,12].

Types of Culture media:

Both Blood Agar and Chocolate agar are routinely used in clinical lab today [98], for example:

a. Chocolate Agar: Blood is heated to 80 °C and used to grow fastidious microorganisms [7].

b. Blood Agar: contains sheep or horse blood + 95% Trypticase soy agar base [7,46].

c. EMB Agar or eosin methylene blue agar is used as a selective for gram negative bacteria for example enteric bacteria [7].

1. Natural Media: a media composed of natural ingredients Bordet-Gengou Agar (Whooping cough) which is a potato based media with glycerol, and is made of natural ingredients that are not chemically defined, and termed complex media [11,12,17].
2. Lowenstein Jensem medium is a selective medium for the tubercle bacillus (T.B.). It contains egg, and is used for growing the T.B. (Tuberculosis) caused by *Mycobacterium tuberculosis.* Whereas *Corynebacterium diphtheria* which causes Diphtheria and whereas may be isolated by Tellurite media [7,11,30,99].
3. Artificial Media- or chemically defined is a medium in which all the ingredients are of known composition [12]. That is all the ingredients have been artificially produced, and added together in known proportions [11,12].
4. Enrichment Medium is a medium that has many nutrients, or growth factors that enhance the growth of a microorganism, It may inhibit the growth of one organism, while promoting the growth of the desired organism or organisms [7,12]. Selenite and tetrathionate broths are both an enrichment for Salmonella [7]. Blood Agar is considered by some as a enrichment medium, since it provides the X (Heme factor). Chocolate agar provides both X & V factor "FAD coenzyme" [7].
5. Chocolate agar may be used as a medium for cultivation of some *Haemophilus* and *Neisseria* species [7].
6. Selective Medium: Enrichment medium that contains a selective agent that inhibits one group of microorganisms, while allowing another group to grow [7].

For e.g. Lowenstein Jensen media is selective for T.B. bacillus (contains malachite a stain) [94]. Bismuth Sulfite agar is a selective medium for the isolation of Salmonella [7,11,12,46,62,94].

McBrides Agar is selective agar for Listeria for dairy products [44]. Moxalactam Agar is selective for *Listeria monocytogenes* but for meats [100]. SS Agar is selective for Salmonella and Shigella Agar [7,12,45,46].

7. Differential medium is a medium that allows more than one type of organism of being distinguished from another, based on reactions that can be macroscopically observed [12]. It may also be described as a medium that contains dyes or chemicals which allow different colony types to be distinguished [7,12,45,46].

An example of a differential medium is Blood agar, with this medium one can distinguish the various types of Streptococci by the type of hemolysis reaction or the lysis of blood cells as alpha, gamma or beta hemolysis [7].

http://www.bacteriainphotos.com/bacteria-photo-gallery.html

http://www.scribd.com/doc/31374502/Microbial-Culture-Media

8. Noncultural microorganism are unable to be grown on routine agar based media. These would include the Viruses, Richettsia (grow on the egg sac membrane), Chlamydia and some of the Spirochaetes, such as the cause of syphilis *Treponema Palladium* [11].

The Rickettsia's are responsible for the Typhus fevers, and the Spotted fevers. *Rickettsia typhi* typhus is contracted by the bite of fleas and is the cause of Endemic typhus. It is referred as murine typhus, and transmitted by a flea. The duration of infection is short lived. *Rickettsia prowazekii* is the cause of Epidemic typhus transmitted by body lice, and infected individuals develop a rash that extends from the trunk, and extends to the extremities [56].

R. rickettsia is the cause of Rocky Mountain Spotted Fever. The disease is transmitted by the tick known as Dermacentor. It is characterized by damage to blood vessels and vessel leakage. It forms a rash primarily on the palms and soles of the feet, and the rash tends to migrate towards the trunk. The major vessel damage is to organs [7,101,56].

http://www.bing.com/images/search?q=Chlamydia+Trachomatis&FORM=IRMHRS

Chlamydia & Trachomatis

https://www.gov.uk/health-protection/infectious-diseases

Various Nonfermenters

Culture Media list and their uses

Below is a list of the most commonly used enrichment, selective and differential agars used in most laboratories today:

a. Eosin Mythelene Blue Agar or (EMB agar) is selective for gram negative bacteria and allows the differentiation of between lactose an non lactose fermenters. It is often used for the selective growth of enteric bacteria [46].

b. MacConkey Agar is also used to differentiate between lactose and non lactose fermenters, and is selective for Gram negative bacteria [7].

c. Mannitol Salt Agar is a selective medium for *Staphylococci* [46].

d. Thayer Martin is a medium for selective isolation of *Neisseria gonorrhea* and *Nesseria meningitidis*, whereas Modified Thayer Martin Agar contains colistin, vancomycin, nystatin and trimethoprim [63,46].

e. Xylose Lysine Deoxycholate Agar or (XLD) are selective for Gram negative bacteria, and can differentiate between lactose non lactose fermenters. It contains three sugars, which are lactose, sucrose and xylose [46].

Thiosulphate citrate bile salts sucrose Agar (TCSB) is selective for the genus Vibrio. It can also distinguish whereas between medium, whereas Noncholera types (those that form green colonies) from cholera causing types. Cholera types that ferment sucrose form yellow colonies on the medium, whereas Noncholera types form green colonies, because they do not ferment sucrose [7]. *Vibrio cholerae* appears yellow on TCSB medium. It ferments the sucrose incorporated into medium [46]. *V. parahaemolyticus* colonies appear green on this medium, because it does not ferment the sucrose is incorporated into the medium [46]. It does not cause cholera, but it may cause food infection from seafood [7,102,103].

http://www.bing.com/images/search?q=Chlamydia+Trachomatis&FORM=IRMHRS

Chlamydia & Trachomatis

https://www.gov.uk/health-protection/infectious-diseases

Various Nonfermenters

http://www.bing.com/images/search?q=Who+Discovered+Vibrio+Cholerae&FORM=IDMHDL

Vibrio cholerae

http://www.scribd.com/doc/31374502/Microbial-Culture-Media

Variety of Culture media used

http://www.sciencebuddies.org/science-fair-projects/project_ideas/MicroBio_Interpreting_Plates.shtml

Interpreting Plates

A List of Bacterial genera

http://www.scribd.com/doc/31374502/Microbial-Culture-Media

Blood Agar, Chocolate, MacConkey & Hektoen Agar

http://www.delta.edu/files/Microbiology/Microbiology.Media.Tests.Pictures.pdf **Various types of Colonies**

Other Various Stains

The **Simple stain** uses one dye does not delineate morphology,

whereas the differential stain consists of more than one dye added in several steps, and the stained structures are differentiated by color as well as by shape [45].

http://classes.midlandstech.edu/carterp/Courses/bio225/Lab%20Stuff/Stains/Bacterial%20Stains.htm

A. simple- one stain only Crystal violet (cv) or methylene blue

B. differential

1. Gram positive: bacteria stain purple (if a bacterium is gram positive)

Gram negative: stains red

2. Acidfast: an organism that stains red. Tuberculosis is acidfast also, both the genus Nocardia and Actinomycetes are acidfast.

3. Nonacidfast: organisms stain blue with the counterstain methylene blue stain [7,81].

https://images.search.yahoo.com/ylt=AwrBT_;_3oDMTB0N-TRvZ2trBHNlYwNzYwRjb2xvA2JmMQR2dGlkA1ZJUDI4MF8x?_adv_prop=image&fr=mcsaoffblock&va=acid+fast+bacteria+mycobacterium

Acidfast Stain

http://www.scienceprofonline.com/microbiology/acid-fast-ziehel-neelsen-bacteria-stain-identify-mycobacteria-nocardia.html

Acidfast bacteria Ziehel Neelsen

Biochemical identification

http://hucmportfolio.pbworks.com/f/Bacterial+Classification+and+Identification.pdf

http://www.bing.com/search?q=Bacterial+identification+-conventional+methods&FORM=MSNH&srch_type=0

http://www.bacteriainphotos.com/biochemical-identification-of-bacteria.html

Biochemical kits

Groups based on Staining methods

2 groups are distinguished one group from another by staining methods.

http://www.scribd.com/doc/17691787/Cultivation-of-Microorganisms

Staining Methods, growth requirements, parameters & colony morphology

http://delrio.dcccd.edu/jreynolds/microbiology/2421/lab_manual/TOC.html

I. **Unstained**

1. A Wet Mount slide, a drop water and coverslip. Are used to observe microorganisms under the microsope [7].
2. Dark field one can observe the Living *Treptonema* Microscopy *palladium* [45].
3. With the Hanging Drop method one uses a concave slide that allows a drop of water with the organisms to hang beneath a cover- slip, above the concave area of the slide, in order to determine whether an isolate is motile [7].

II. **Stained**

1. Allow the shape, size and structure of an microorganisms. Staining helps to make Microorganisms more Observable [2].

III. **Negative staining**

With negative staining, staining only occurs up to the border of the microorganism. The cell wall has a negative charge (-) and the stain has a negative charge (-) charge, so the stain just coats but doesn't actually stain. An example is using India ink for capsules. *Cryptococcus neoformans* (the cause meningitis) has a capsule that can be seen by using the India ink stain [2,45,104].

IV. **Differential stain** is where two or more dyes stain different parts of an organism. An example below:

1. Gram stain is a differential stain that helps to distinguish two groups of microorganisms based on their ability to either retain the primary stain crystal violet (stain purple), and or be decolorized by alcohol treatment, and stain red by the counterstain safranin [45].
2. Acidfast: Acidfast is also a differential stain in which a smear is gently heated while being stained with carbonfuchsin until the stain is driven in. A acid alcohol is used to decolorize the slide, and then a methylene blue is appplied as a counterstain. Those isolates that stain red with carbonfuchsin are considered acidfast, whereas those staining blue are nonacidfast. Examples of acidfast bacteria are Mycobacterium (M.T.) *Mycobacterium tuberculosis* and *Mycobacterium leprae*, the cause of leprosy [6,45].
3. Special stains include the endospore, flagella, capsule, and stain for the storage compounds (volutin or polymetaphosphate) [45].

A endospore stain is when a heat fixed smear is steamed and malachite green is applied to drive the stain into an endospore. A second stain safranin is now applied for approximately for one minute. The result is that if a endospore is present, it should retain the malachite and turn green, whereas the vegetate portion of the cell will be stained red by the safranin [2].

The staining of capsules with nigrosin or India ink is frequently used for the capsule of *Cryptococcus neoformans* (cause of meningitis), which can occur in cerebral spinal fluid. A smear is, but is not heat fixed but is allowed to air dry after applying made with India ink. Capsules appear as though a light halo surround the cell. Capsules may be polysaccharide, polypeptide, polyphosphate, and may prevent phagocytosis [2,7,45,104,105].

4. Volutin or metachromatic granules may be stained by by methylene blue, and they appear red. It is believed to serve aa a food reserve and or energy source [42,7].

Special selective - methods

Fastidious Microorganism two examples would be *Neisseria*

meningitides and N. gonorrhoeae. Neisseria gonorrhoeae are Gram negative diplococci that occur in pair with adjacent sides flattened. Chocolate Agar - will grow both these fastidious species. Both Martin Lewis and Thayer Martin are selective for Neisseria. Thayer Martin agar differs from Martin Lewis in that nystatin has been substituted for anisomycin and the level of vancomycin was increased from 3 to 4µg/l [61,94]. Both Martin Lewis and Thayer Martin are selective for both Gonorrhea and *N. meningitides*. Both organisms may be identified by API, BactiCard, and the Gonochek 11 System, identification by carbohydrate fermentative is discouraged [61,94].

http://www.bing.com/images/search?q=neisseria+chocolate+agar&qpvt=Neisseria+Chocolate+Agar&qpvt=Neisseria+Chocolate+Agar&FORM=IGRE

Many Chocolate Plates with the Gonococcus & *Neisseria meningitides* nonmotile.

http://www.austincc.edu/microbugz/handouts/Stain%20protocols.pdf

http://classes.midlandstech.edu/carterp/Courses/bio225/Lab%20Stuff/Stains/Bacterial%20Stains.htm

Various stains - gram, acidfast & endospore

http://www.scribd.com/doc/17691787/Cultivation-of-Microorganisms

https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/ThayerMartinModified.htm

Neisseria & Moraxella species Corynebacteria, Streptococci

http://en.wikipedia.org/wiki/Thayer-Martin_agar

Neisseria species

Immunologic Methods

Immunological Methods test for the presence of Microorganisms by using specific antibody's, or serological methods. Serology is the study of serum or more specifically the use of Immunologic assays to detect antibodies for the purpose of diagnosing an infectious disease [29].

Visualization Methods

Staining Methods

1. Unstained bacteria a) wet-mount b) dark field Microscopy (test for motility).

b. Darkfield microscopy is often used to observe microorganisms such as *Treptonema palladium* (Syphilis organism) which is very difficult to stain normally [45].

Microorganisms are objects as small as 0.2 micrometers (µ), look below for the variety of sizes they can occur: for E.g. Poxvirus (100 nanometers or 0.1µ) [2]. RBC'S average 7-8µm, *Echerichia coli*=1.5µ, *Staphylococcus aureus* = 1.0µm or micrometer [2,7,106].

Ways to observe Microorganisms

Representative gram stain morphologies

Some pathogenic microorganisms appear gram negative (GM-) when observed under the microscrope, while others like Staphylococci appear as gram positive cocci in clusters. The Streptococci also have a morphology like Staphylococci but may form chains. The genus Neisseria also forms cocci but in pairs or diplococci. Whereas the genus Clostridium is a gram positive bacillus and stains purple. *Escherichia coli* is a gram negative bacillus rods, that appears red after counterstained with safranin [7].

http://en.wikipedia.org/wiki/Acid-fast

http://faculty.ccbcmd.edu/courses/bio141/lecguide/unit1/prostruct/afcw.html

Other types of Microscopic examination

Darkfield Microscopy and Electron Microscopy

Some Mycobacteria are considered atypical because the type health treatment needed is not like tuberculosis (T.B.) [7]. With the genus Mycobacterium, Mycobacterium intercellularium complex, and *Mycobacterium bovis* (Bovine tuberculosis). *Mycobacterium bovis* BCG strain is used as the vaccine that is used today. M. bovis occurs in animals and man. The Tubercle bacillus (T.B.) is acidfast and stains red, if whereas the counterstained with methylene blue appears blue if nonacidfast [7,18,99].

http://faculty.ccbcmd.edu/courses/bio141/lecguide/unit1/prostruct/acidfast.html

http://en.wikipedia.org/wiki/Acid-fast

Immunology

Over the last few decades, science has progressed because of advancement's in microscopes, microscopic techniques, and primarily because of better ways of understanding the genetics of the double helix. The emergence of the cell sorter in the late seventies, and new improvements in cell lines (Hela, hybridoma), monoclonal antibodies, and better tissue typing methods, have also made transplants more readily available [7,29,36,45,107-110].

With the development of better instrumental methods, such as, DNA hybridization, Rapid Agglutination (latex) tests, DNA fingerprinting, Gene probes, Ribotyping, Polymerase Chain Reaction (PCR), and Fluorescent Antibody Techniques, Medical diagnosis and treatment have become more rapid, effective, and highly specific. With so many advances in the sciences, one can only briefly highlight the advances that have been made in just the last twenty years. From DNA fingerprinting and human genetics, what was once thought to be science fiction has now become a scientific reality [36,108,111].

The cell sorter which originated many years ago gave researchers a valuable tool for working with identical cell

types (Clones of T-lymphotocytes). It laid the groundwork to manufacture pure cells (monoclonal antibodies) in large quantities, and made possible the reality of more accurate, faster, and reliable diagnostic tools [36,107].

Scientists today have also been consciously working to have a better understanding how the immune system works collectively, as a whole. In that effort, they have been interested in how immune cells interact with each other. Within the last several years, scientists have begun to have a more in-depth understanding of how cells communicate with each other. They understand that cells communicate with each other by releasing immunological factors, (the cytokines) that regulate the immune system's response to foreign substances such as, antigens, allergens and autoimmune diseases. With a greater understanding of these factors and, their interaction, they are now potentially able to harness the immune system, to destroy unwanted microorganisms and conquer disease. In the future, scientists will be able to use immunological methods to pinpoint many microbial diseases, cancer, and reduce the devastation of autoimmune disease [36,108,111,112].

http://uhaweb.hartford.edu/BUGL/immune.htm

Humoral, Cellular & Innate Immunity

The heart of the Immune system lies within the Stem cells, which originate from the bone marrow, and are essential to regenerate blood related cells. The granulocytes (various white blood cells) originate from stem cells, as do the B-cells. B-cells also differentiate in the bone marrow into mature B-cells, whereas other stem cells give rise to lymphocytes, and Special B and T lymphocytes [50].

B lymphocytes differentiate into mature cells, in the bone marrow, and may become plasma (antibody producing) cells, that function in Humoral Immunity. T lymphocytes, however, must migrate to the thymus, where they differential under the influence of hormonal factors, and where they become mature T-cells, and are involved in "cellular immunity" [50].

Regardless of cell type, shortly after birth our body labels cells as self or non-self by receptors [50]. Both cell types, either self or non-self, are recognized by the immune system, by the presence of receptors on their surface [49]. When cell receptors make contact with an antigen, certain cytokines are released, and this aids phagocytic cells (white blood cells) to recognize the antigen, and destroy it within a phagocytic vacuole [37]. The body can thus distinguish foreign cells, and set up an immune attack against various antigens, such as, bacteria, fungi, viruses, and all considered non-self [37,49,50].

http://www.cancerhelp.org.uk/about-cancer/what-is-cancer/body/the-immune-system

Immunity, active & innate and T & B lymphocytes

Granulocytes stain readily with wrights stain and arise in the bone marrow, and become the basis for the formation of the red blood cells, and the White cells (the eosinophiles, polymorphonuclear leukocytes, and basophils). The agranulocytes, however, differentiate in the thymus, and supply the body with the majority of lymphocytes, monocytes and macrophages [7,37].

Each type of granulocyte on the other hand, performs a prominent function in eliminating microbes by phagocytosis, which helps wounds heal, whereas the lymphocytes (agranulocytes) are involved in other immune reactions, such as inflammatory reactions, such as tissue rejection, and autoimmune disease. Lymphocytes are also capable of recognizing the presence of malignant cells. Over the course of much investigation and scientific inquiry, scientists have acquired a better understanding how immune cells talk to each other. They have used this new knowledge to improve the success rate of tissue transplantation, conquer more diseases, and perpetuate weapons that pinpoint cancerous growths, and improve the quality of life.

Recently a new diagnostic tool was been discussed in the discover last year. A "small pill-like tablet" was discussed in Discover magazine as a means to fight melanoma, a skin disease that is 85% fatal [50]. The tablet contains proteins biopsied material isolated from a mouse tumor. The device is porous, and is made of a plastic that's biodegradable. It also has the benefit of GMC-SF (granulocyte macrophage colony-stimulating factor). The tablet cells once able to recognize tumor cells, they are able to enter the lymph nodes, and begin to attack the tumor [113]. Experimental mice have been seen to have a 40% reduction in the tumor [50,113].

http://uhaweb.hartford.edu/BUGL/immune.htm

http://uhaweb.hartford.edu/BUGL/immune.htm#intro

Introduction to the Immune System

The Immune System

The Immune System generates immune cells from the bone marrow, which undergo further development, into the various immune cells, which function together, or independently during an immune response. These cells may release chemical factors (cytokines) that enhance an immune reaction, at the molecular level. Cytokines may also trigger molecules that booster the severity of inflammatory reactions, or may enhance a immune reaction against foreign invaders, and cancer [50,114].

Most white blood cells enhance phagocytosis, whereas certain lymphocytes (T-lymphocytes) are involved in allergies, transplant reactions, and even autoimmune disease (cytokines, interleukins involved). The immune system continuously produces ample numbers of red blood cells, white blood cells, and lymphocytes, which may protect the body from various microbes, tumor cells, and various autoimmune diseases [7,50,115].

http://uhaweb.hartford.edu/BUGL/immune.htm#intro
http://uhaweb.hartford.edu/BUGL/immune.htm#blood

The Fungi

Fungi are microorganisms that occur singularly or multicellularly and are characteristically eukaryotic. They also lack chlorophyll and their cell walls contain chitin. Reproduction is primarily asexual, by extension, of elongation as well as branching of its main components namely its hyphae. Therefore elongation of the hyphae is a form of asexual reproduction, in which there is vegetative elongation, or branching of the filaments or hyphae [7,31].

Fungi Nutrition & Parasitism

http://www.mycolog.com/

http://www.mycology.adelaide.edu.au/

In general, most fungi have crosswalks or septa, however, some lack fungal hyphae cross walks or septa that separate adjacent cells, and the hyphae are said to be coenocytic (lack septa). The hyphae are arranged in a continuous outgrowth, and thus form what is referred to as a Mycellium [31]. Metabolically fungi have three modes of obtaining their nutrition, chemoheterotrophic (require preformed organic compounds, saprophytic (live, dead debris), or parasitic and infect other organisms [7,31].

Asexual Reproduction

Asexual Spores are formed by sporulation, by budding or fragmentation. There are three major types of vegetative spores which arise from specialized cells [7,31,116].

1. Blastospores- are a product of budding
2. Chlamydospores-are round thick walled cells formed directly from hyphae.
3. Arthrospores-are barrel shaped spores that arise from aerial hypha [7,31].

Aerial Sporulation

The Zygomycetes may be characterized by forming a sac-like structure called the sporangium, and their asexual spore the sporangiospore. Sexual spores or zygospores are formed by the union of opposite mating types [7,31,116]. Aerial conidia are spores that are borne from spore bearing structures called the conidiophores. The conidia may be formed in chains, by budding (blastoconidia), and or by fragmentation of septate hypha (arthroconidia) [7,31,107].

Another class of fungi the Ascomycetes form their spores within a sac or ascus. An example of a ascomycete would be the genus Aspergillus. Aspergillus forms its spores or conidia from conidiophores, and may arise from supporting structures called the sterigmata. Both Aspergillus and Penicillin are ascomycetes, and may be characterized by the fact that their conidia are formed at the terminal swelling or phialide [7]. Some fungi occur as filaments or may appear as yeasts, yeast like of form filaments in hyphal agar media, for example the yeast like fungus would be *Trichosporon beigelii* [7,31].

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

Outline of Fungi

Methods for direct examination

1. In the Slide Culture technique, a small square of sterile agar is inoculated with teased hyphae, and then placed in a humidified petri dish. The fungi may in this way be observed as the fungus sporulates [116].

2. With a Wet Mount a slide is used and exposed to KOH treatment. It is a method that can provide a quick look for filaments and or fungal spores [116].

3. India Ink (capsules), Lactophenol aniline cotton blue are stains used to observe a fungus and its filaments, and or its spores. The stain colors the spores and filaments and also kills the fungus [7,31,117].

Fungal Classes

A. Fungi have been historically classified into Four Classes, namely the:

1. Zygomycetes form coenocytic hyphae and form sporangiospores, An example is Rhizopus species [116].
2. Ascomycetes the Sac fungi form ascospores in a sac, an example is Aspergillis [7].
3. Basiomycetes are the Club fungi since they form clubshaped spores. An example is *Cryptococcus neoformans* which causes meningitis [7].
4. Fungi Imperfecti or Deuteromycetes and would be for example, both the genus Alternaria, and Cephalosporium.

Molds are also be classified based on where they found in the body [118,119].

The term Mycoses refers to the disease that a fungus may cause. The examples include:

1. Cutaneous is involved in the skin, hair and nails "Dermatophytes" [7,116,120].
2. Subcutaneous involves the subsurface or just below the skin or Chromoblastomycosis caused by Sporotricium [7,116,120,121].
3. Systemic is an infection that can be found in all parts of the body, without any preference (usually Lung involvement) e.g. Coccidioidomycosis caused by Coccidioides immitis [7,107,111,112].
4. Opportunistic a secondary infection resulting usually from an underlying condition is called "Candiasis", [116,120,121].
5. Superficial is only confined to the outer extremity of the hair, hair follicle, or inside the hair itself [116,120,119].

Both Black piedra is caused by Piedraia hortae, whereas White piedra is caused by Trichophton ovoides, and *Trichophyton inkin* are superficial infections [120]. In rare cases, the saprophytic fungi may cause disease, particularly when debilitated individuals are involved [120]. *Aspergillus* and *Rhizopus* species can cause a pulmonary disease, if the patient is immune deficient [116,120].

However the most serious infections are caused by the Systemic Fungi, in which their primary infection generally occurs in the lungs. "Valley Fever" is the cause of Coccidiodomycosis. *Coccidioides immitis is* the cause of Valley Fever, and is endemic to the San Jonqoin Valley. The majority of infections do not result in Symptoms [116]. The infective spores are arthroconidia primarily infective, and infections are primarily within the lungs. However infections in the lungs can spread to other organs, and may result in deteriotion of bones, joints, as well as the liver and spleen. It may also lead to meningitis infections. There are about 100,000 cases of coccidioidomycosis each year, with between 50-100 deaths annually [116,120,121,122].

The interesting aspect of the systemic fungi is that they can exist in two forms. They are said to be dimorphic, that is they grow as a mold at room temperature and a yeast at 35 °C. They can survive below the soil, if the environmental conditions are with elevated soil temperatures [116]. At body temperature when the infective spores (arthrospore) are inhaled, it may set up an infection in the lungs. However, in the lungs, the arthrospores enlarge become round and form what are referred to as a spherule. If spherules rupture in the lungs, they can release the endospores which can be phagocytized, and enter the lymphatics and blood system. Thus the endospores can spread to other sites, and set up a secondary infections. The arthocondia are the infective phase, with anthrospores converting to spherules within the lungs. Within approximately four days spherules release endspores within the lungs. Each spore is capable of repeating the cycle of infecting the alveoli [104,116,119-122].

Histoplasma capsulatum is endemic to the Ohio Valley area. It is often referred to as "Darling's Disease". In nature it occurs as a mold when grown at room temperature, but will convert at 37 °C in the laboratory to the yeast phase. The infective spores are inhaled into the lungs [7,116,120-122]. It primarily is localized in the lungs, but may be disseminated through the Reticuloendothelial system (RES), that consists of phagocytic cells, such as macrophages that make up the liver, the spleen, and bone marrow that respond in a immune response. It is noted microscopically for its "tuberculate macroconidia" during the mold phase [7,116,120-123].

Blastomyces dermatitidis is also a dimorphic fungus, that is found associated in the Mississippi Valley. It causes a disease primarily again in the lungs, but can cause "cutaneous lesions." The yeast forms are noted for their broad base buds [116,120].

Paracoccidioides brasiliensis is the agent of South American Blastomycosis, and is named so since it is edemic to South America and particularly Brazil. Its key characteristic is its yeast phase that exhibits multiple buds with a narrow neck [116,120].

Sporothrix schenckii is a dimorphic fungus that is usually caused by a wound puncture. It is commonly referred to as "Rose Thorn Disease," since it is usually accompanied by the puncture of a rose thorn. The disease is called Sporotrichosis, and my cause nodular lesions of the cutaneous and subcutaneous. The yeast phase can be grown in the laboratory at 35 °C, and may characteristically appear as yeast-like and refered to as "Cigar bodies" [116,120].

http://www.mycolog.com/fifthtoc.html

The Fifth Kingdom

Algae

Algae are eukaryotic microorganisms that may be unicellular or multicellular. In some cases that may form chains of cells that form filaments. This large group of microorganisms lack roots, stems and leaves found in plants [31]. The larger multicellular algae may form the body of the algae or thallus. The thallus generally contains holdfasts (often branched), that anchor the alga to rock. They also have stem-like or hollow stipes and leaf-like blades. Algae lack connective tissue, such as xylem and phloem, and therefore absorb nutrients from water throughout their surface. Algae are able to keep themselves upright in their water environment, by a gas-filled bladder called a pneumatocyst [7,31].

Algae reproduce asexually by simple cell division, and may at times alternate between asexual and sexual reproduction. The majority of algae are photosynthetic, but there are a small number of chemoheterotrophic types. Algae contain chlorophyll a as their energy gathering pigment. There are several phyla of algae, and each have a characteristic pigment. The Brown algae are considered kelp and belong to the Phaeophyta, whereas the red algae are members of the phylum Rhodophyta [31]. The Green algae like the other two Phyla contain cellulose in their cell walls. The Dinoflagellates and Water Molds also have cellulose in their cell walls, whereas the Diatoms contain pectin and silica. Algin a product from Brown agar is used in ice cream, rubber tires, and hand lotion. Red algae are used as a thickener in evaporate milk, ice cream, as well as a solidifying agent in bacterial culture media [7,31].

http://botany.si.edu/projects/algae/classification.htm

A general description of the various phyla of algae

http://delrio.dcccd.edu/jreynolds/microbiology/2421/lab_manual/TOC.html

Biochemical, Culture, Procaryotic & Eukaryotic Manual

Eukaryotes in detail

The Eukaryotes

The Fungi

Over the last 10 years, the incidence of serious fungal infections has been increasing. These infections are occurring as nosocomial infections, due in part to people with compromised immune systems [31].

Fungi may be beneficial. They are important in the food chain because they decompose dead plant matter. They recycle vital elements, through the use of extracellular enzymes such as cellulases. Fungi are the primary decomposers of the hard parts of plants, which cannot be digested by animals. Nearly all plants depend on symbiotic fungi, known as mycorrhizae, which help their roots system to absorb minerals and water from the soil [31].

Fungi are also valuable to animals. Fungi-farming ants cultivate fungi that break down cellulose and lignin from plants, providing glucose that the ants can then digest. Fungi are used by humans for food (mushrooms) and to produce foods (bread and citric acid) and medicines (alcohol and penicillin). There are over 100,000 species of fungi, with only about 200 pathogenic to man and animals. The study of fungi is called mycology. All fungi are chemoheterotrophs, requiring organic compounds for energy and carbon. Fungi are aerobic or facultatively anaerobic; only a few anaerobic fungi are known [31].

Vegetative Structures

Fungal structure

Fungal colonies are described as vegetative structures because their cells are involved in catabolism and growth. The molds and fleshy part of the fungus is called the thallus (body) of a mold. It consists of many hypha, that are joined together by its filaments or hyphae (singular; hypha). Hypha can grow to immense proportions. The hyphae of a single fungus may extensive that can reach miles in length [31].

With most molds the hyphae are considered septate, with cross-walls separating adjacent cells. These cross-walls are called septa (singular: septum), which divide them into distinct uninucleate (one-nucleus) cell-like units. In some fungi, the hyphae contain no septa and appear as long, continuous cells, with many nuclei. These hypha are called coenocytic hyphae. Even in fungi with septate hypae, there are usually openings in the septa, that make the cytoplasm of adjacent cells continuous. These fungi are actually coenocytic organisms, too. In a sense adjacent cells are always in communication with its neighbors [31].

Reproduction

Hyphae grow by elongating at the tips. Each part of a hypha is capable of growth, and when a fragment breaks off, it can elongate to form a new hyphal filaments. Growth in most cases begins at the tips of the hyphae by their elongation. The portion of a hyphae that obtain nutrients is called the vegetative hyphae, whereas the portion concerned with reproduction is the reproductive or aerial hypha. So named because they project above the surface of the medium on which the fungus is growing. Aerial hyphae often bear reproductive spores. When environmental conditions are suitable, the hyphae grow to form a filamentous mass celled a mycelium, which is visible to the unaided eye. The vegetative hypha are primarily responsible for maintaining nutrition, whereas the aerial hypha are responsible for the formation of reproductive structures [31].

http://www.uwlax.edu/biology/volk/fungi3/sld001.htm

Mycology a Slide Show

Yeasts diversity

Yeasts are non-filamentous, unicellular fungi that are typically spherical or oval. Like their counterparts the molds, they are found throughout the environment. Frequently they are found as coating many fruits and leaves. Many yeasts like Saccharomyces reproduce by budding.

When the parent cell is about to bud, it will form a small bud on its outer surface. As the bud elongates, the parent cell's nucleus divides, and the nucleus will be divided equally between the mother cell, and the new bud forming. Cell wall material will be portioned to each cell, and the bud eventually breaks away [31].

When buds that are formed do not detach themselves, they may form chain of buds called pseudohyphae. *Candida albicans* is an example of a yeast that forms pseudohyphae. *C. albicans* does form a pseudohyphae and has been found to be more tissue invasive, when forming pseudohyphae [31].

Yeasts may grow facultative anaerobically or they may grow aerobically, with oxygen as the final electron acceptor. This provides the yeast with the flexibility of growing either aerobically or facultative anaerobically. This is a valuable attribute because it allows these fungi to survive in various environments. If given access to oxygen, yeasts perform aerobic respiration to metabolize carbohydrates to carbon dioxide and water, denied oxygen they ferment carbohydrates and produce ethanol and carbon dioxide. The ability of yeasts to grow facultative or aerobically gives them versability to grow either in the presence or absence of oxygen [31].

Dimorphic Fungi

Some fungi, particularly the pathogenic species, exhibit a dimorphism or two forms of growth. At 25 °C the fungus exhibits a mold like form and producing both vegetative and aerial hypha, whereas at 37 °C it exhibits a yeast like form, and reproducing by budding. Dimorphism in pathogenic fungi is temperature-dependent, at 37 °C the fungus is yeast like, and at 25 °C, it is mold-like. Therefore the convertion of one phase to another is temperature dependent [31].

Life Cycle

Some filamentous fungi are capable of reproducing by fragmentation of their hyphae. They may also form spores by both sexual and asexual reproduction. In general fungi are identified based on the spore type and microscopic appearance [31,124].

Fungal spores, differ from bacterial endospores. Bacterial endospores are characterized by their ability to overcome extreme conditions of the environment. A endospore gives rise to one single bacterial cell, whereas fungal spores are considered reproductive, since they give rise to a new cell. Fungal spores may not survive extended periods of adverse conditions, as compared to the ability of endospores which have been found to survive even long periods of less than favorable conditions [31].

The majority of fungi forms asexual spores from aerial hypha. They produce different types of asexual spores depending on the species. Fungi may produce spores either asexually or sexually. Asexual spores are formed by the hyphae of one organism, and as the spores germinate, these spores are genetically identical to the parental cell. Sexual spores differ from asexual spores in that they are formed by the union of opposite mating types, from the same species of fungus. Asexual spores undergo mitosis and cell division, but there is no fusion of nuclei from cells [31].

There are primarily two different types of asexual spores that may be produced by a fungus. One type is called a conidiospore or conidium, which is unicellular or multicellular. This spore is not formed in a sac, and may form spores in chain, when produced from the end of the conidiophores. The conidia of Aspergillus are produced in this manner. Other conidia may be produced through the fragmentation of hyphae, forming what is called the thick walled arthroconidia. One example of a fungus that reproduces by anthroconidia is the fungal species, *Coccidiodes immitis*. Another type of conidium is called the blastoconidium, where conidia are formed from the budding of its mother cell (parent cell). Spores formed from budding are often formed from a yeast such as Candia albicans, and Cryptococcus species [31].

Another conidial type is the chlamydoconidium which is considered a thick walled spore, often thought as a resting spore. *C. albicans* is a fungus that produces chlamydoconidia [7,31]. An additional spore formed by some is the sporangiospore, which is formed within a sac, and called the sporangium. The sporangiospore is formed at the ends of the aerial hyphae. Each sporangium may give rise to hundreds of sporangiospores for ex. Genus Rhizopus [31].

Sexual Spores

Sexual Spores are produce by sexual reproduction, and undergo three phases as depicted below:

1. Plasmogamy. Occurs when the cytoplasm of one haploid nucleus fuses with the cytoplasm of another nucleus type.
2. Karyogame. The nuclei from opposite mating types fuse to form a diploid zygote.
3. Meiosis. It is reductive division, in which the diploid condition is reduced to a haploid condition (haploid 1N). There may be the formation of recombinates [31]. The sexual spores produced by fungi characterize the phyla. In laboratory settings, most fungi exhibit only asexual spores. Consequently, clinical identification is based on microscopic examination of asexual spores [31,104]

Nutritional Adaptations

Fungi are chemoheterotrophs, and capable of utilizing preformed organic compounds by absorption of nutrients [7].

1. Fungi for the most part grow best in an acid pH (about 5), which inhibits the growth of most common bacteria. They grow in the presence of air, whereas yeasts are facultative anaerobes. Fungi also are capable of growing more readily under greater osmotic pressure.
2. Other attributes are their ability to grow under conditions of lower available water, as compared to bacteria which are unable to grow under those conditions. Fungi may grow with lesser nitrogen as compared to their bacterial counterparts, and are able to ultilize a wide variety of complex substrates as complex carbohydrates [11,31].

Medically Important Phyla Of Fungi

Zygomycota

They are a group of fungi that are aseptate or coenocytic, and are often associated as food contaminants. An example of a zygomycete is *Rhizopus stolonifer*. *R. stolonifer* is often referred to as the common bread mold [31]. Rhizopus produces black sporangiospores, which are thick walled and are formed from the fusion of identical mating types. Rhizopus may reproduce sexually with the fusion of nuclei of opposite mating types, and giving rise to the sexual zygospore [31].

Ascomycota

The Ascomycota are called the sac fungi, since they form their ascospores within a sac like structure called the ascus. They are primarily septate molds, but also include some yeast forms. Conidia are often referred to as dust, since the spores seem to spread like a powder in the wind. The sexual spore or ascospore is formed by the fusion of, like or unlike nuclei, and the spores formed witin a ascus [7,31].

Basidiomycota

The Basidiomycota are a group of septate fungi. They include the mushrooms, puffballs, stinkhorns, and smut fungi. They have received the common name club fungi, because the basidium is said to be club shaped. Each basidium will form four basidospores, which arise from the exterior of the basidium. Some basidiomycota are also capable of producing asexually conidiospores. Fungi that produce both asexual and sexual spores are described as telemorphs, whereas fungi that only capable of producing asexual spores are referred as anamorphs [31].

An infection by a fungus is called a mycoses. Infections that are long term are referred as chronic, although there are many different types of mycoses such as: superficial, cutaneous, systemic and opportunist. The Mycolic infections that are confirmed to the epidermis, hair and nails are referred to as cutaneous mycoses caused by dermatophytes. They are confined to those areas (hairs, skin and nails), since they ultilize the keratin protein found in those tissues. Transmission from man to man to animal to man is achieved by direct contact [31].

Superficial mycoses is usually confined to the hair surface or epidermal cells. Opportunist mycoses predominantly occurs with individuals who are immune compromised, or have experienced some type of tissue trauma [7]. Pneumocytis is an example of an opportunist mycoses, since it may cause a mycotic infection as a result of AID's or antibiotic treatment. The fungus Stachbotrys has been associated with damaged and leaking roofs, where the fungus may get a foothold due to home damage. The genus Mucor under the right circumstances may cause an opportunistic infection, particularly with diabetic patients, leukemia, or undergoing immunotherapy. Aspergillus, Penicillium and Cryptococcus may cause fatal infections with debilitated individuals, but usually does not affect the immunocompetent [31,119,125-127]. Both Rhizopus and Mucor also may cause a Mucomycosis or an opportunistic infection that occurs mostly in patients that are susceptible due to immune-incompetence [31,125].

Yeast Infections

Probably one of the most common causes of urinary tract infections is caused by the yeast Candida albicans,. It is also a common cause of the infections of the mucous membrances. It is a common cause of throat infections called thrush. The infection may also be referred to as Candidiais, and is often associated in newborns, AID's patients, and may occur following antibiotic therapy [31].

Aspergillosis is also an opportunistic mycosis, it is caused by the genus Aspergillus. This disease occurs in people who have debilitating lung diseases or cancer, and have inhaled Aspergillus spores. Opportunistic infections by Cryptococcus and Penicillium can cause fatal disease in AIDS patients. These opportunistic fungi may be tramsmitted from one person to an uninfected person, but do not usually infect immunocompetent people [31,119,125].

A yeast infection, or candidiasis, is most frequently caused by Candida albicans, and may occur as "vulvovaginal candidiasis" or thrush, a "mucocutaneous candidiasis." Candidiasis frequently occurs in newborns, in people with AIDS, and in people being treated with broad-spectrum antibiotics. In 1993, Andrea and Donald Stierle saved the yews by discovering that the fungus Taxomyces produces taxol. Taxol is a treatment for breast cancer. *Candida oleophila*, can be and is used to prevent undesirable fungal growth on harvested fruits. This process of biocontrol works because the *C. oleophila* grows on the fruit surface before spoilage fungi grow [31].

http://www.mycolog.com/

Parasitology

Parasitology is the study of parasitic protozoa and worms. Parasitism is one where there are two or more organisms living together. One is the host in or upon which the parasite lives

[118,128]. A endoparasite is a parasite that lives inside the host, such as ticks, lice, fleas, mites, bedbugs and etc [105]. Obligate parasite is a parasite that needs a host thoughout its life Cycle [7,118,128].

A Facultative parasite is a parasite that can survive (throughout its life Cycle), with or without its host [128]. An example is Naegleria and Acanthameba (occur streams, and pools). They have an affinity for the Central Nervous System of Man and can cause a high Mortality rate [118,128,129].

The parasitic organisms are described by the general term Helminth's. The Helminth's include the trematodes (flukes), cestodes (tapeworms) and Nematodes (roundworms). They are unique in that they are bilaterally symmetrical. That is, they have left and right halves that are mirror images of each other [31,118].

In the Phylum Protozoa, there are 5 subphylum.

The subphylum Sarcomastigophora is characterized by containing organisms that have either pseudophodia or flagella.

The Super Classes: Sarcodina contain the amoebae move by pseudopodia, whereas the Mastigohpora possess flagella [7,31].

Protozoa usually are macroscopic and are considered animals since they are usually without cellulose walls or chlorophyll [130]. As a group their method of locomotion is by pseudopodia. They range in size from two micrometers to centimeters in diameter. There are 2 major forms of Intestinal Amoeba [7,31].

The Active invasive feeding form is called the trophozoite (troph). The other form is called the Cyst form, and is the resistant "immobile stage." This stage is the form that is often passed from the parasite, in order that others might be infected [131].

Superclass Sarcodina

Family Entamebidae are characterized by their nucleus type. They are characterized by the type nucleus in which the nucleolus (Endosome) is "granular" in appearance. The endosome may be a "Compact granular endosome", or it may be broken up into a series of granular elements. Entamoeba range in size from 10-60µm [129].

The Number, shape and position of the endosome is systemically important. There are several genera that are noteworthy in the family Entamebidae, namely the *Entamoeba histolytica, E. nana, E. coli, Iodamoeba butschlii, Dientamoeba fragilis,* and the nonintestinal amoeba Naegleria, Hartmanella, and Acanthamoeba [131].

Entamoeba histolytica is an amoeba that lives in the gut and is able to dissolve tissue. It is primarily parasite of the Large intestine of man, and as such, lives off mucus, and red blood cells. Microscopically, the organism is characterized by its centrally located endosome, and peripheral radiating spokes of chromatin. It feeds by both diffusion as well as phagocytosis. It also has contractile vacuoles. It is pathogenic, because it can damage the intestinal wall [131].

It does form a cyst, which also has a central endosome, but may have up to 4 endosomes. In addition, it will form chromatoidal bars with rounded ends. The chromatoidal bars are associated with RNA and protein.

Entamoeba coli differs from *E. histolytic* in that its endosome is eccentric and has numerous vacuoles in its cytoplasm, and the chromatin bars are more closely around the nucleus. It primarily feeds off bacteria and digested materials. Its size is around 75µm. The cyst of *E. coli* has 8 nuclei, and Jagged shaped chromatid body. *E. nana* is 6-10µm in length and forms a "elongate shaped cyst, with 1-4 nuclei/cyst [7,131].

The *E. coli* trophozoite is uninucleate and somewhat elongated. It forms a very large irregular endosome, that may or may not be associated with chromatin strands. Key characteristics:

1. Some instances, it is completely void of any chromatin material, between the endosome and nuclear membrane. It is found also in the large intestine.
2. It is characterized by an "irregular nucleus."
3. May have the presence or absence of chromatin strands, and tine endosome [7].

Iodamoeba butschlii is a common parasite of individuals. When it is stained with iodine it gets its name from the "bluish colored vacuole." The vacuole is stained due to the presence of glycogen. With *I. butschlii* usually there is no chromatin material around the nucleus. The nucleus varies in shape, and its structure in relationship to the nuclear membrane. However, it is often recognized by its characteristic "flower basket nucleus arrangement" [7,132].

Dientamoeba fragilis is characterized by its two nuclei, which consists of 4 endosomes, 'separate" granular endosomal parts. It is found in the Large intestine. It is very distinct looking, since forms a binucleate troph form. It does not form a cyst [128].

The Family Harmanellidae include the 3 genera, Naegleria, Hartmanella and Acanthamoeba. These amoeba are able to penetrate the mucus membranes of the nose and throat. They have an affinity for the Central Nervous System, they literally destroy brain tissues [131].

Protozoan Diseases

Giardiasis

Giardia lamblia infects the small intestine, the disorder is called giardiasis. When cysts are ingested from fecal material, they pass through the stomach and small intestine, and the trophozoites are released from cysts in the colon. Eventually the trophozoites attach to the colon, where they feed on mucus. They cause symptoms of inflammation of the bowel, dehydration, weight loss, and nutritional deficiencies which are common in infected children. They also interfere with absorption of fat-soluble vitamins, and resulting in copious amounts of diarrhea [7,131].

Giardiasis is fecally transmitted through the engestion of contaminated food, water and fecally contaminated hands. The cysts are not killed by ordinary sewage treatment and chlorination. The organism has two sucking disks, a axostyle, and 4 pairs of tailing flagella [133].

Balantidium coli

Balantidiasis unlike the previous parasites it is a ciliate. It is the only ciliate that causes human disease. It is distributed worldwide, and particularly in the tropics. The organism is transmitted by cyst in fecal matter. After the cysts are engested, they rupture and release trophozoites, which now are able to invade the walls of the large intestine. The disease is known as "balantidiasis". Symptoms are similar to amoebic dysentery. Balantidiasis can be fatal if it perforates the large intestinal wall, and leads to fatal peritonitis. Pigs serve as a reservoir of infection, therefore contact with feces should be avoided [7,129,133].

Cryptosporidium

Cryptosporidium is a cause of opportunistic infections worldwide. It is often transmitted from kitten and puppies. The organisms live in or under the membrane of cells lining the digestive and respiratory systems. In 1993 over 400,000 residents of Milwaukee, Wisconsin were infected by *Cryptosporium latum*. The organism was being pulled into the Waste Water Treatment Plant. However the plant was unable to kill the infecting cysts. The municipal water company which was down stream of the waste water treatment plant, received the contaminated water, and since they lacked a filtration system, they were unable to remove the cysts (oocysts) from the treated drinking water [7,133].

Cyclospora

Cyclospora also produces oocysts which are spread via the oral-fecal route. It is believed that between 10-100 oocytes are all that are necessary the initiate an infection. The symptoms include: flu-like symptoms, watery diarrhea, bloating, anorexia, abdominal pain, weight loss and etc. Diagnosis is the finding of oocytes in feces [131,133].

Helminth & Gastrointestinal Diseases

Fluke Infections

Fasciola hepatica (the sheep liver fluke) produce egg's that are taken up by snails and the larva become cercaria within the snail. The cercaria leave the snail and encyst on vegetation (bamboo shoots) as metacercaria. When humans eat raw, undercooked vegetation, the metacercaria excyst and may bore thru the intestinal wall and liver. It penetrates the liver capsule and gets into the biliary ducts, and feeds on liver tissue. *F. hepatica* causes a severe condition in the liver, leading to cirrhosis and death [133,134].

Clonorchis sinensis the "Chinese liver fluke" is widely distributed in Asia. *C. sinensis* differs from *F. hepatica*, in that it requires two intermediate hosts. The first host is the snail where a miracidium is transformed into a cercaria. The cercaria penetrates between the scales of fish, and become a metacercaria. When man eats undercooked fish, he may engest a metacercaria and become infected. The adult flukes find their way to the liver, where they sometimes perforate and damage the liver [134,135].

Fasciolopsi buski, is common in pigs and humans in the orient. It lives in the small intestine and causes chronic diarrhea and inflammation. It causes a "verminous intoxicatin," an allergic reaction to toxins in the fluke's metabolic wastes [134,135].

Tapeworm Infections

Humans are often infected by eating poorly or undercooked pork. The "pork tapeworm" *Taenia solium* can reach a size of 2-7 M, whereas the beef tapeworm *Taenia rhynchus saginatus* is between 5 to 25 M [31,136]. With Taenia the egg has striations, and one can see those striations between outer envelope. An egg or oncosphere is eaten by a pig, the oncosperes are carried by the blood stream to the muscles, and other tissues. The oncospheres develop into the cysticercus the (bladder worm). When man eats under cooked pook, he engests the cysticercus. A invaginated scolex (tapeworm head) attaches to the small intestine, and gives rise to a adult tapeworm. Man can be both a intermediate and definitive host. Hogs and wild boares may serve as intermediate hosts, while man often serves as the definitive host. The oncospere is striated and contains hooks, and therefore is said to be armed. When the ova are engested, viable larvae can develop into adult tapeworms. When adult tapeworms develop in the intestine, they may absorb large quantities of nutrients, and lead to malnutrition. They may form large masses of ribbonlike worms which may can blockage of the intestine [31,122].

Larva may attach to the intestine and become an adult tapeworm. Adults in the small intestine may cause malabsorption, since they absorb excess amounts of nutrients. The can cause intestinal blockage, if they reach massive numbers. Autoinfection occurs when the egg covering disintergreting, larva may penetrate the intestinal wall. The released larva enter the blood, and may migrate to various tissues and develop into a cycticercus or bladder worm [133,128].

Both the pork and beef tapeworms have identical life cycles. The only difference is that the intermediate host is cattle. Cattle acquire the tapeworm by engesting the embryonated eggs from the soil. The larva from the oncosphere hatch from the egg and penetrate the intestina wall and enter blood vessels, and becom cysticerci in the muscles. Man acquires the cysticercus by eating under cooked beef. The cycticercus disintegrates and the inverted scolex grows, and becomes a adult tapeworm [128,131,133,136].

Echinococcus granulosus

When humans are frequently in contact with dogs, they can be infected by the tapeworm *Echinococcus granulosus*. The eggs of this tapeworm produce cysts called hydatid cysts. The cysts, can contain hundreds of tiny immature worm heads and often reach the size of a grapefruit or larger, and can exert pressure on organs. When a cyst ruptures, it can cause a severe allergic reaction such as anaphylactic shock [31,131,137].

Family Hymenolepepididae- all have Cysticercoids as larvae.

Hymenolepis nana is called the "dwarf tapeworm. All genera of this family use an intermediate host, usually an arthropod (insects, lice, flour beetles). The oncosphere or hexacanth embryo comes out of its membranes, and develops a cysticercoid, which later becomes an adult in the intestines. These tapeworms cause diarrhea, abdominal pain, and convulsions [131].

Diphyllobothrium latum this tapeworm requires two interemediate hosts, namely the copepod and the fish. It forms what is referred to as a coracidium, the first larval stage. It has

numerous cilia and so it can swim. It is next engested by the copepod and the larval stage becomes a Procercoid within the copepod. It next is transformed into a plerocercoid in the musculature of its host (a fish). In its secondary host, and becomes a fully developed tapeworm. This tapeworm can be up to 30 feet in length and can be responsible for vitamin B12 deficiencies [131,133].

Trichinosis

Trichinosis is caused by the small roundworm called *Trichinella spiralis*. The parasite usually enters the digestive tract as encysted larvae in poorly cooked pork, venison and meat from the other game animals. In the intestine cysts release larvae that develop into adults. The adults mate, the males then die, and the females produce living larvae before they too, die. The larvae migrate through blood and lymph vessels to the liver, heart, lungs, and other tissues. When they reach skeletal muscles, especially eye, tongue, diaphragm, and chewing muscles, they form cysts. The formation of cyst in humans represent a dead-end for the worms, as they will not be eaten and passed on to another host. Encysted worms remain alive and infectious for years [31,128,138].

These parasites cause tissue damage as adults and as migrating and encysted larvae. The adult females penetrate the intestinal mucosa and release toxic wastes that produce symptoms similar to those of food poisoning. Wandering larvae damage blood vessels, and the tissues they enter. Death can result from heart failure, kidney failure, respiratory disorder, or reactions to toxins. Freezing does not necessarily kill encysted larvae, and microwave cooking is safe only if the internal temperature of the meat reaches 77 °C [31,128,138].

Hookworm Infections

The two main organisms causing hookworm are *Ancylostoma duodenale* and *Necator americanus* (11 to 13mm). Eggs in feces quickly hatch in moist soil. There the eggs release free-living larvae that feed on bacteria and organic debris, grow, molt, and become mature parasitic larvae. If larvae reach the skin, typically of the feet or legs, they burrow through it to reach blood vessels and carry them to the heart and lungs. The larvae then penetrate the lung tissue, and some are coughed up and swallowed. In the intestine the larvae burrow into villi and mature into adult worms. The adult worms mate and start the cycle over again. When in the lungs, there they cause many tiny hemorrhages, but the greatest damage is to the lining of the entire small intestine. They are responsible abdominal pain, loss of appetite, protein and iron deficiencies [134,139].

Ascariasis

Individuals are infected by ingesting food and water contaminated ascaris eggs. Once in the intestine, the eggs hatch and larvae penetrate the intestinal wall, and enter the lymph vessel and venules [71]. Although the larvae can invade and cause immunological reactions in almost any tissue, most move through the respiratory tract to the pharynx and are swallowed. Larvae move to the small intestine, mature, and begin to produce eggs. Larvae burrowing through the lungs can cause hemorrhage, edema, and blockage of the alveoli. Their toxic wastes can elicit allergic reaction, and with sufficient numbers may cause intestinal blockage and sometimes perforation [134,139,140].

Toxocara species is associated with cats and dogs (puppies), when humans are infected by larvae. Visceral larva migrans the larvae of the parasite migrate through such organs as the liver, lung, and brain, where they cause tissue damage and allergic reactions [141].

Trichuriasis

Trichuriasis is caused by the "whipworm". It is estimated that 300 million people are infected. When eggs are swallowed, they hatch as. juveniles which crawl into enzyme-secreting glands of the intestine called crypts of Lieberkuhn, where they develop. They return to the intestinal lumen (central space), where they reach full maturity within months of initial infection [134,138].

The Adult whipworms can damage the intestinal mucosa and feed on blood. They cause chronic bleeding, anemia, allergic reactions to toxins, and susceptibility to secondary bacterial infection [134,138].

Strongyloidiasis

Strongyloidiasis is caused by the parasite called Strongyloides stercoralis. This parasite is unusual in that females produce eggs by parthenogenesis, that is without fertilization by a male. Adult females are about 2.2 millimeter long by 0.04mm wide, attach to the small intestine, burrow into underlying layers and release eggs containing noninfective larvae. Many eggs hatch in the intestine and are passed with the feces. In soil, larvae can become free-living adults, or develop into infective larvae and penetrate the skin of new hosts [138].

Infective larvae, which penetrate the skin are carried by blood to the lungs. There they bore their way to the trachea, travel to the pharynx, and are swallowed. When they arrive in the small intestine, they develop into adults, and restart the life cycle. They can cause a secondary bacterial infection in any tissue and can lead to septicemia [138].

Pinworm Infections

The parasitic organism that causes pinworm is called *Enterobius vermicularis*. Like the hookworm, this parasite can complete its life cycle without an alternate host. Adult pinworms attach to the epithelium of the large intestine and mate, and the females produce eggs. Egg-laden females migrate towards the anus during the night, release their eggs on the exterior of the anus, and then crawl back in [31,134,138].

Engested eggs hatch in the small intestine, and release larvae that mature and reproduce in the large intestine. Some may migrate upward to the stomach, esophagus, and nose. Pinworm infection is not debilitating but can cause considerable discomfort, and interfere with normal nutrition. Large numbers can cause the rectum to protrude. Pinworms are diagnosed by finding eggs that have been released overnight, or upon rising early in the morning. A tongue depressor is attached to a piece of transparent cellophane in the anal area, and any eggs should stick to the cellophane tape [31,134,138].

Schistosomiasis or Bilharziasis

Schistosomiasis is one of the most dreaded, and most debilitating diseases of the world. These are the Blood flukes

of man and related creatures, which are found in the intestinal mesentaries of the hepatoportal vein, and the bladder [142,143]. They have only one intermediate host, namely the aquatic snail. Eggs when laid have fully developed miracidum, and within two weeks produce Cercaria (larvae). These larva are called Furcocercarium because it has a bivercate or "forked tail" [142,144].

When bathing in infected waters, man becomes infected when the bivercate-cercaria penetrate the skin. Once the cercaria penetrate the skin the forked tail is lost, and the larva enter the capillary system. The tailless cercaria make their way thru the heart, lungs, (bladder) into the portal system, and finally in three weeks grow to maturity, in their species specific area [142,143]

Schistosoma mansoni, *S. japonicium* and *S. intercalatum* all are found in the Mesenteric Veins, that drain the large and small intestine. Whereas the species *S. haematobium* has an affinity for the bladder veins. The major damage is due to the eggs and not by the adults. *S. mansoni,* most of these organisms are widely distributed in Western Hemisphere, Puerto Rico, and Southern Brazil. *S. mansoni* its ova has a strong lateral spike, whereas *S. haematobium* has a terminal spike. *S. japonicium* has a weak lateral spike [31,134,142,143].

Filarial Worms

Wucheria bancrofti is caused by the name of the filarial worm called "Elephantiasis". The organism is transmitted by the Blackfly mosquito. The Sausage form is the form found in the mosquito. The infective larva for man is the filariform larva. Upon penetrating the skin through the bite wound from the black fly, the larva pass to the lymphatic vessels and nodes, where they grow to maturity in 6 or more months [131,141,143].

The adult worms tend to frequent the varices of the lymphatic vessels of the lower extremities, the groin glands and epididymis in the male, and the labial glands in the female. The microfilariae migrate from the parent worm through the walls of the lymphatics to the neighboring small vessels, and are carried by the lymphatic circulation to the blood stream. However, the microfilaria larva are found in their greatest number in the lungs during the day. Whereas when the carbon dioxide is low, (during the night), the microfilarial larva are found primarily in the peripheral blood. The Major damage is precipitated by the Adults, due to their blockage of the lymphatic system. This blockage of lymph results in the enlargements in the area of the groin, legs and feet [131,141,143].

http://www.tulane.edu/~wiser/protozoology/guide.html#outline (Study Guide)

Parasitology Atlas

<http://www.dpd.cdc.gov/DPDx/HTML/Image_Library.htm>

(Parasitic image Libary)

Conclusion

In summary, microorganisms are found just about everywhere on earth. They are found deep in the earth, many miles down. From the coldest to the hottest, saltest as well as deep in the ocean (near as the tectonic plates) [8,20-22]. Microorganisms seem to have the uncanny ability to occupy any environment, whether hostile or non-hostile. They may be freeze dried (lyophilized) and later be thawed out, and then divide and grow with proper nutrition [12,145]. Even NASA has had problems with the persistence of microbes, hitching a ride on some of their space vehicles. Microorganisms have been found in their space craft assembly facility at the Kennedy Space Center in Florida. They also were found in drinking water, and even circuit boards used for a mission to Europa. The majority of these organisms are contaminates occurring as sporeformers (endospores), capable of resisting gamma rays and cosmic radiation [146,147]

Not only are microorganisms everywhere to be found in the natural environment, also live on us, around us and within use. They can have a detrimental effect on us, and with those instances when they can cause disease. They cause disease by forming toxins (endotoxin and exotoxins) and various enzymes that can affect various tissues and organs [28]. Our bodies try to prevent these pathogenic microbes from gaining a foothold in our bodies, by a number of white blood cells (basophiles, eosinophiles neutrophiles), dendritic cells, monocytes and macrophages. They are able to travel as invaders, and try to overcome our natural body defenses and immune system [112].

Our white blood cells as well as lymphocytes (B & T) seek out and try to destroy any microorganism entering the body. These cells are constantly being replaced by the bone marrow [37]. They arise from the bone marrow, and give rise the precursor of cells the stem cells [37,50].

The plasma cells are derived from the B lymphocytes, differentiate and become mature cells within the bone marrow. The T lymphocytes are also are derived from the bone marrow, but must migrate to the thymus in order to undergo maturation there. Fully matured plasma cells when confronted by an antigen, must undergo cell division, and reproduce in order to produce antibodies specific for the antigen that stimulated their formation. T lymphocytes on the other hand are involved in tracking down cancer cells, and involved in hypersensitivity (allergies) and autoimmune disease. Therefore, our immune system is composed of a series of white blood cells (granulocystes) and lymphocytes (agranulocytes), which together work to prevent disease [37,43,50].

In 1951 a young woman arrived at the medical clinical at John Hopkins Clinic in Baltimore. She had a small lesion on her cervix and was examined by her physician. She was treated with radiation. Her cervical cells still showed the unusual cell division vigor, even after she had been treated with radiation several times. Her cervical cells continued to grow in tissue culture, even after much exposure to radiation. Unfortunately, this young college student did die from her cervical cancer, but for some reason her cells still live on today. Her name was Henrietta Lacks. She is still remembered, even today, though she passed away soon after her several clinic visits. Her cells still live on even today, in the cell culture lines called Hela cells, and are routinely used to support the growth of many viruses in cell culture cell [7,148].

It would be years before the importance of these Hela cells would become noticed by the medical community, and their

importance. Finally, somehow someone from a laboratory recognized this cell lines importance. By chance someone fused a plasma cell with and myeloma cell, and the most unique think happened. The two cells fused. The newly fused cell was able to produce antibodies, but more importantly of only one type. This new fused cell would be called a hybridoma cell, producing only one type antibody redirected by its plasma cell, and now known as a monoclonal antibody [149]. With the development of monoclonal antibodies, it became clear that this new cell type could lead to the development of many diagnostic tests, that were not possible before [31,50,149].

Today there are rapid Streptococcal tests for the various Lancefield groups of Streptococci (for e.g. PathoDX using (monoclonal antibodies), as well as agglutination tests for Salmonella somatic O antigen, *Staphyloccous aureus* (latex agglutination) and may be used for identification of other microorganisms as well [68,150,151]. A two day precipitate has been developed for the food Microbiology laboratory. Precipitate test samples can be screened as negative within two days. The immunoprecipitate band that is formed from positive Salmonella samples, is similar to a horseshoe shape [152]. Other immunological methods that have been used to recognize microorganisms by using immunoelectrophoresis. Immunoelectrophoresis is used to detect various antigen antibody reactions. Antigens and antibodies are allowed to migrate across a thin agar gel. A small electric charge is applied and charge particles migrate across the field. Those antigens that are recognized by their antibody form a precipitant band or band of identity, while those not forming a recognized band is called a band of non-identity. In this way proteins my be separated based on their antigen antibody reactions in an electric field [7,43]. Another test that is frequently used in the microbiology laboratory is the Fluorescent Antibody Technique (FAT). In the Fluorescent Antibody Technique a specific antibody for a antigen (or organism) is bonded with a fluorescent dye or fluor [7]. When the antigen reacts with the specific antibody, it can be seen directly with a Fluorescent Microscope, because the antibody will bond with the organism, under the ultraviolet light (uv). When the organism with the fluor antibody are exposed to uv light, it can be seen as a bright fluorescent color with the aid of the fluorescent microscope [7,43].

When culturing microorganisms, they must be supplied the necessary nutrients they need for growth. In order to grow microorganisms scientists have developed culture media for that purpose. There are many types of culture media, some are specifically designed to provide the essential needs of that particular group of microorganisms, or specific type of microorganism. Most labs try to encourage the growth of one group of organisms over another, whether gram negative bacteria (Enteric bacteria), the Streptococci, Staphylococi and other fastidious types of organisms like Haemophilus and *Neisseria meningitidis* [11,41,143,150,153].

In the case of the Enteric bacteria and particularly enrichment media like GN broth or selenite cysteine broth have been used for many years, to facilitate the growth of Salmonella and Shigella. There are other broths that are often used in the Microbiology, Brucella broth for Campylobacter, LIM broth for Streptococcus agalactiae (Group B Streptococci) which causes neonatal meningitis), Brain Heat Infusion Broth, Campylobacter broth and Listeria enrichment broth for *Listeria monocytogenes* [143,150,154].

The general purpose media used in the laboratory today is sheep blood agar. It will grow a variety of microorganisms from gram positive to gram negative microorganisms, as well as yeasts and molds. Blood and Chocolate agar are both nonselective but will grow even more a variety of microorganisms. Chocolate agar will allow a number of fastidious microorganisms such as, Hemophilus, Neisseria species, Streptococci, and Nonfermentative bacteria to be isolated in culture. Culture media like xylose deoxycholate agar (XLD) and Hektoen Enteric agar (HE agar) are specially formulated for the recovery of gram negative bacteria, particularly the enteric bacteria like Salmonella and Shigella. Other types of culture media like Campylobacter and Brucella agar have been implemented for the more fastidious microorganisms such as Campylobacter, and the genus and Brucella species. Tellurite Blood agar is often used for the selection for Corynebacterium diphtheria (cause of diphtheria) [11,47,77,79,143,150,153,155-157].

Culture media have been devised for the growth and selective culture of fungi as well. Corn meal agar is used for the recovery of chlamydospore forming *Candida albicans*, whereas Mycosel or Mycobiotic agars with the addition of cycloheximide and chloramphenicol will grow either saprophytic or diphasic molds. Even more important brain heart infusion with the addition of cycloheximide and chloramphenical may be used for the selective isolation of dimorphic fungi [116,158]

Culture media therefore may be used as a enrichment for a particularly organism or organisms. It may facilitate the growth of one group of microorganisms, while inhibiting another. As is the case with the use of GN broth, while coliform bacteria are inhibited, Salmonella and Shigella are not. Culture media may have the addition of additives that for example lower the pH, and thus facilliate the growth or yeasts and molds (pH 5 or less), or by adding certain antibiotics in order to make culture media selective for a particular microorganism [11]. The formulation of culture media therefore is an elaborate detailed intricate scientific undertaking by itself [11,41,156].

Microorganisms are stained in order to be observed microscopically. Once they have been gram stained. The gram stain slide is observed under the microscope for the size, shape and morphology. Once that has been established, they may be subcultured to a broth and or another culture medium. Isolates may be cultured for additional tests, or inoculated to an additional culture media in order to maintain purity of an isolate [11,28,45].

In general, once a microorganism has been isolated in culture, some additional types of conventional tests may be required. Along with a gram stain, additional tests like the oxidase, catalase and motility may need to be performed. Besides the microscopic and culture appearance, isolates my need to be worked up biochemically and identified [11,28,45,153].

Microorganisms that express a typical gram stain and

morphological characteristics are identified by convention and by rapid methods where available. Conventional methods of identification, usually involve the ability of a microorganism is to utilize a number of substrates. For each substrate that an organism capable of using, can change the color of the pH indicator, indicating each substrate that is utilized. The generated biochemical pattern will then be compared to the standard biochemical patterns of known microorganisms, for a identification [28,41,80].

The book by Macfaddin offers not only places similar microorganisms in groups, but also lists a number of biochemical tests that may help for an identification. This book is primarily for clinical isolates, but provides flow diagrams for the identification of gram positive microorganisms, enteric's, gram negative bacteria and nonfermentative bacteria [80,93].

Lehman et al. in Chapter 15 provides a number of conventional tests that may be used to identify the Group A, Group B, Viridans Streptococci, and the Enterococci [159]. An excellent manual for clinical microbiology has been written by M.K. York. The manual is strictly for Clinical microbiology, however, it does provide the types of culture media needed for specimen collection from various body sites. The manual also describes the appearance of colonies on a variety of culture media, and key conventional tests to be performed for a identification. The most important aspect is that the manual has flow diagrams for both the gram positive and gram negative microorganisms for identification. Dr. Fung's Handbook lists a variety of instruments, and methods for the rapid identification of microorganisms. Dr. Fung also offers a ten day workshop on rapid methods as well [90,160].

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