UNIT – 3 Taxonomy

Principles of Biological Classification

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Taxonomy is the science of classifying organisms.

1. Classification

The orderly arrangement of living things into groups on the basis of their similarities is known as classification.

2. Nomenclature

The process of naming organisms in a logical manner is known as nomenclature.

From 'nomen' = Latin for name.
Oldest names have preference and two organisms should not have the same name. Also, names of living persons should not be used.

3. Identification

Comparison of an unknown organism (*eg*. bacterial strain) with the characters of known ones up to species level to achieve identification is known as identification.

4. Cladism

The study of the evolutionary relationships between organisms is known as cladism.

Organisms are classified into any particular group because they have certain common characteristics.

Importance of Classification of organisms--

1) It establishes the criteria for identifying organisms;

2) It arranges related organisms into groups based on shared characteristics; and

3) It provides important information on how organisms evolved.

A classification system based on collecting individuals into groups and groups into progressively more inclusive and broader groups is called a **hierarchical scheme** of classification. A basic principle of taxonomy is that members of higher-level groups share fewer characteristics than those in lower-level groups. For example, nearly all bacteria have a cell wall, but in some the wall is Gram Positive (G+) and in others it is Gram negative (G-).

Microbial Classification Approaches -

With changing history of biology microbial classifications have been attempted with different approaches. These are as follows:

i) Artificial approach by **Aristotle**

ii) Natural approach by **Carolus Linnaeus**

iii) Phylogenetic approach by **Haeckel, Whittaker, Woese**

iv) Adansonian approach by **Adanson**

v) Macromolecular approach

i) Aritificial Approach of Biological Classification by Aristotle –

The earliest systems of biological classification were artificial. **Aristotle**, during fourth century B.C. classified the living organisms into two kingdoms the **Animals and the Plants**. This division was simply based upon

a) ability of active movement and
b) ability to photosynthesis. The microbes were put in both these kingdoms

ii) Natural Approach by Carolus Linnaeus (Binomial Nomenclature)

In the middle of 18th century **Carolus Linnaeus** (1758) devised a **binomial nomenclature** system that was more useful. This system expressed the biological nature of the objects that it classified; there was very little knowledge of microbes in this system also.

Binomial Nomenclature

A Swedish biologist **Carolus Linnaeus** devised a taxonomic scheme that was both practical and adaptable to expanding information.

Carolus Linnaeus introduced a binomial nomenclature (each organism is designated by 2 names). **The first name is the organism's genus name & the second is its specific nickname**. **Together, the 2 constitute the species name**. The species name is always Latinized and underlined or Italicized but not underlined. The first alphabet of genus name is written in capital letter, but the first alphabet of specific nickname is not written in capital letter. Thus, the proper designation for humans is **Homo sapiens (or *Homo sapiens*)**.

For convenience, the genus designation can be replaced with an **initial** if the complete genus name has been used recently enough to avoid possible confusion. For example, the bacterium *Staphylococcus aureus* becomes *S. aureus*. All eukaryotes & prokaryotes are named in this way but the viruses are not named in this way.

The Linnaean scheme uses the following hierarchical classification scheme:

Kingdom

Phylum (division)

Class

Order

Family

Genus

Specific epithet (nickname)

Example: Humans Example: *Treponema pallidum* (causes syphilis)

Kingdom – Animalia Kingdom- Monera (Prokaryotae)

Phylum – Chordata Phylum- Gracilicutes

[Subphylum - Vertebrata]

Class – Mammalia	Class -Scotobacteria
Order – Primates	Order - Spirochaetales
Family – Hominidae	Family -Spriochaetaceae
Genus – *Homo*	Genus -*Treponema*
Species - *Homo sapiens*	Species – *Treponema pallidum*

iii) Phylogenetic Approach by Haeckel, Whittaker and Woese

The post **Darwinian** biologists thought in terms of evolutionary affinities between organisms. In the 19th century the concept of a natural system accordingly changed to phylogenetic approach. However, this approach needs a better knowledge of fossil records of organisms. A German biologist, **Ernst H. Haeckel** separated the **unicellular organisms from plants and animals, placing them under a third kingdom, Protista**.

❖ The Three kingdom Classification System (Haeckel's 3 kingdom concept)

1. **Kingdom Plantae:** – eukaryotic; multicellular; cell wall present; sexual and asexual reproduction; photosynthetic; acquire nutrients from environment by absorption.
2. **Kingdom Animalia:** - eukaryotic; multicellular; *no cell wall;* primarily sexual reproduction; acquire nutrients from environment by ingestion (some parasites by absorption).
3. **Kingdom Protista: -** The fungi, protozoa, bacteria and algae were included in this kingdom.

❖ The Five Kingdom Classification System (Whittakar's 5 kingdom concept)

No single classification system is completely accepted by all biologists, but one of the most widely accepted is the **five-kingdom system.** A modem system of this type is devised by **R. H. Whittaker** (1969). The

microorganisms have been included in the kingdoms Monera, Protista and Fungi in this system.

1. **Kingdom Plantae:** – eukaryotic; multicellular; cell wall present; sexual and asexual reproduction; photosynthetic; acquire nutrients from environment by absorption. E.g. All plants.
2. **Kingdom Animalia:** - eukaryotic; multicellular; *no cell wall;* primarily sexual reproduction; acquire nutrients from environment by ingestion (some parasites by absorption). E.g. All animals.
3. **Kingdom Monera:** – prokaryotic; unicellular; most have a cell wall; reproduction usually by binary fission; photosynthetic, some chemosynthetic; acquire nutrients from environment by absorption. E.g. Bacteria
4. **Kingdom Protista:** – eukaryotes; most are unicellular (some are organized into colonies); cell wall present in some, absent in others; reproduction mostly asexual, sometimes sexual; some are photosynthetic; acquire nutrients from environment by absorption and ingestion; this group includes the algae (resemble plants), the protozoa (resemble animals), and the euglenoids (resemble both plants and animals).
5. **Kingdom Fungi:** – eukaryotic; unicellular or multicellular; cell wall present; sexual and asexual reproduction; acquire nutrients by absorption of organic matter and from dead organisms. E. g. Molds, Yeasts, Mushrooms

❖ The Three-Domain Classification System (Woese 3 domain concept)

After the discovery of the archaeobacteria in the 1970's, scientists suggested that these organisms represented a third cell type and they proposed another scheme for the evolution of living things from a universal common ancestor. This common ancestor gave rise to the archaeobacteria, the urkaryotes, and the eubacteria (true bacteria). They hypothesized a group of urkaryotes that gave rise to the eukaryotes directly rather than by way of the prokaryotes.

In 1990 **Woese** suggested a new taxonomic category, the **domain**, to be erected above the level of kingdom. The three domains Woese proposes are-

i) The domain Eukarya - contains all those kingdoms of eukaryotic organism (animals, plants, fungi, and protists). The traditional kingdom Monera has been divided into 2 domains:

ii) The domain Bacteria - (true bacteria)

iii) The domain Arachaea -The Archaea exhibit many differences from the Bacteria:

- Different cell membrane structure
- Cell wall present, but not composed of peptidoglycan
- First amino acid in proteins not methionine like in other bacteria and eukaryotes
- DNA Contains histone-like proteins similar to eukaryotes (true bacteria have no histone proteins)
- Live in only extreme environments (groups include extreme halophiles, extreme thermoacidophiles, and methanogens)

iv) Adansonian Approach - Numerical Taxonomy

This was first suggested by a French biologist, **Michel Adanson** in the 18th century. Here the taxonomic arrangement is based upon quantification of the similarities and differences among organisms. There are used as many characters as possible, each of which is of an equal weight.

It is assumed that if each phenotypic character is given equal weighting, it should express numerically the taxonomic distances between organisms, in terms of the number of characters they share, relative to the total number of characters examined. The results are fed into a computer so as to define the similarities and differences between microorganisms and thus to indicate possible natural groupings.

Example of numerical taxonomy (classification)

For simplicity, this example uses only 10 characters for 5 strains (or **OTUs** - Operational Taxonomic Units).

Characters are expressed as positive (**+**) or negative (**-**) depending whether an organism possesses a particular feature or not.

Some characters cannot be used for certain organisms being investigated. In this case '**NC**' (No Comparison) can be recorded.

CHARACTER OTUs (STRAINS)	**A B C D E**
1. GRAM STAIN	+ + + + +
2. ROD SHAPE	- - + - +
3. SPORES	+ + - + -
4. MOTILITY	+ - + - -
5. CATALASE	- - - - +
6. OXIDASE	+ + - + -
7. INDOLE	- - - - -
8. ACID FROM GLUCOSE	- + + - -
9. GROWTH AEROBICALLY	+ + + + +
10. VP TEST	+ - - + -

Each OTU (strain) is compared in turn with every other OTU and the number of **matches** calculated. Both positive and **negative** matches are scored. In other words, if two OTUs both show a character, this is a positive match. If neither of two OTUs show a character, this is a negative match. If one OTU is positive for a character, and their OTU is negative for the same character, this is **no match** and is not scored. Simple matching coefficients are calculated (usually in percent) as follows:

Percent Simple Matching Coefficient (**SMC%**) =
Sum of positive matches + Sum of negative matches x 100

Total number of tests - Number of tests which are NC

In the above example for OTUs A and B,
the **SMC%** = 4 + 3 x 100 = 70%
Total number of tests - Number of tests which are NC 10 - 0
In other words, the two strains A and B share 70% of the characters tested. Matching coefficients of 75% or greater indicate that the two strains are likely to be the same species.

The higher the matching coefficient, the more closely related the two strains. High values suggest that the strains should be put in the same group (taxon) or closely related groups.

Of course, the method is only as good as the characters chosen and Numerical Taxonomy can never be truly objective. Having calculated all the matching coefficients, a similarity matrix can be constructed:

OTUs OTUs (STRAINS)

	A	**B**	**C**	**D**	**E**
A	100				
B	70	100			
C	50	60	100		
D	90	80	40	100	
E	40	50	70	50	100

The blank spaces represent redundant similarity coefficients. The 100% figures should be obvious!!! Listing the values of OTU pairs in order, scanning and construction of a dendrogram come next. Numerical taxonomy is very time consuming and was not popular until the 1960s when it was revived. Why???

Summary

1. Perform tests on different OTUs (strains). At least 60 independent, unit characters must be used.
2. Calculate simple similarity coefficients for each OTU with every other OTU in the group.
3. Construct similarity matrix.
4. List OTU pairs in descending order of coefficient value.
5. Scan at set intervals.
6. Construct dendrogram.
7. Analyse dendrogram.

v) Macromolecular Approach –

The above mentioned approaches are based mainly on the phenotypic traits. The growth of molecular biology has now opened up a number of new approaches to microbial taxonomy. For identification and classification of bacteria, a range of criteria are now used. Some of the criteria are represented. The shape and size of organism, its oxygen, pH and temperature requirements, and laboratory culture characteristics are considered. Photosynthetic ability, capsule production, staining reaction, spore forming ability and type of movement are other characteristics: Biochemical digestion of certain carbohydrates, proteins and fats are also considered. There are certain techniques used that give near information of the genetic characters of organisms.

The identification of an organism is the process of determining its species. Various characteristics of the organism are determined by appropriate observations and tests, and these are then compared with published descriptions of the various species. Identification of microorganisms is done on the basis of

1. Morphology
2. Cultural characteristics
3. Biochemical characteristics
4. Nutritional requirements
5. Physiological properties
6. Serology
7. DNA base Composition
8. Nucleic Acid Hybridization
9. 16s rDNA sequence analysis

1. Identification of microorganisms on the basis of Morphology.

The **size, shape and arrangement** of cells are determined by microscopic examination of stained smears. Examination of living organisms in a hanging drop preparation shows motility, if present. Non-motile organisms frequently display *Brownian movement,* a motion caused by the molecular activity in the fluid. Motility can also be determined by other methods, for example growth on semisolid agar which permits migration of the organism away from the line of inoculation.

Organisms are differentiated on the basis of various **staining reactions.** Among the more frequently used staining methods are the Gram stain, Acid fast stain, Flagella stain, Capsule stain, Spore stain, Negative stain, and the stains for various intracellular granules such as metachromatic granules, iodophilic granules (starch or glycogen-like substances), and sudanophilic granules (poly β-bydroxy-butyrate). The Gram stain is of great value, because organisms are classified as Gram positive or Gram negative. The staining reactions, however, vary with the composition of medium, conditions of growth and the age of the culture.

2. Identification of microorganisms on the basis of Cultural characteristics.

Pure cultures of organisms can be studied by growing them on a wide variety of culture media. Under appropriate cultural conditions, organisms

show characteristic types of growth, and such observations are useful in the identification of the organism. Growth characteristics are commonly observed on the following types of culture:

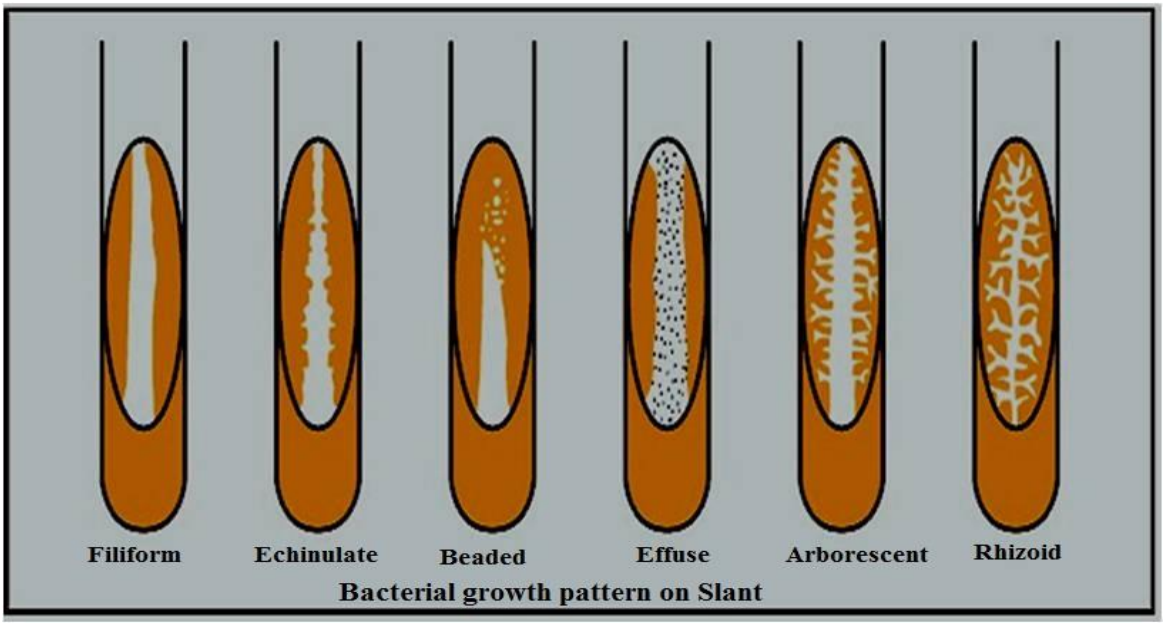
a. Colonies on solid media (plate cultures).
b. Growth in liquid media.
c. Growth on agar slants.
d. Growth in agar stabs.
e. Growth in gelatin stabs.

Description of colonies on solid media should include **size, shape, color, surface / margin, elevation, reflected and transmitted light / opacity, texture / consistency, appearance etc**.

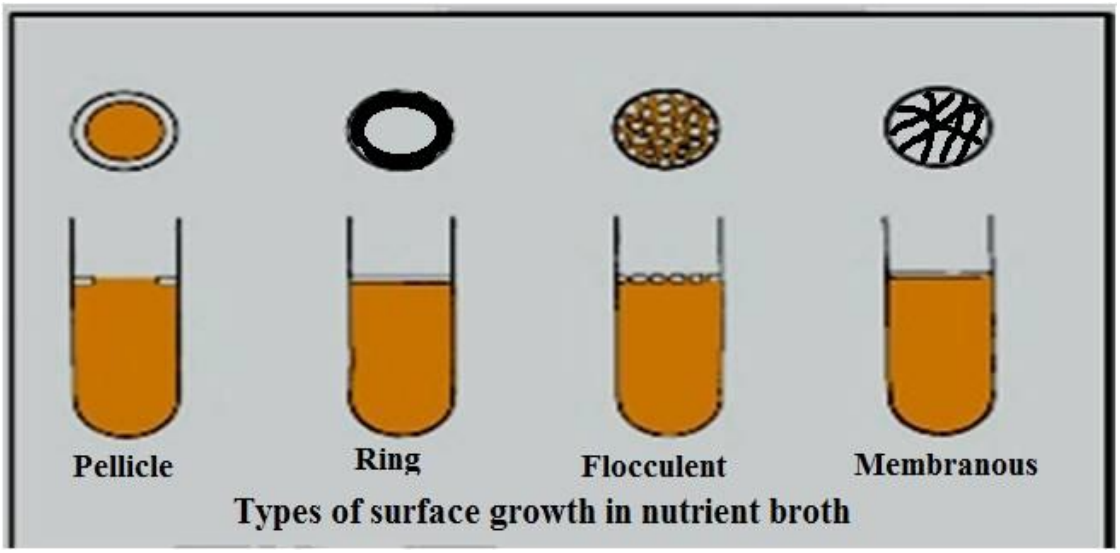
Similar information can also be secured from agar slants and agar stab cultures. Growth in broth may consist of uniform turbidity, or cloudiness, sediment at the bottom of the culture, or a membrane or pellicle on the surface.

A gelatin stab may show the type of growth as well as the type of liquefaction. These growth characteristics are frequently typical of certain species, but are modified under different cultural conditions.

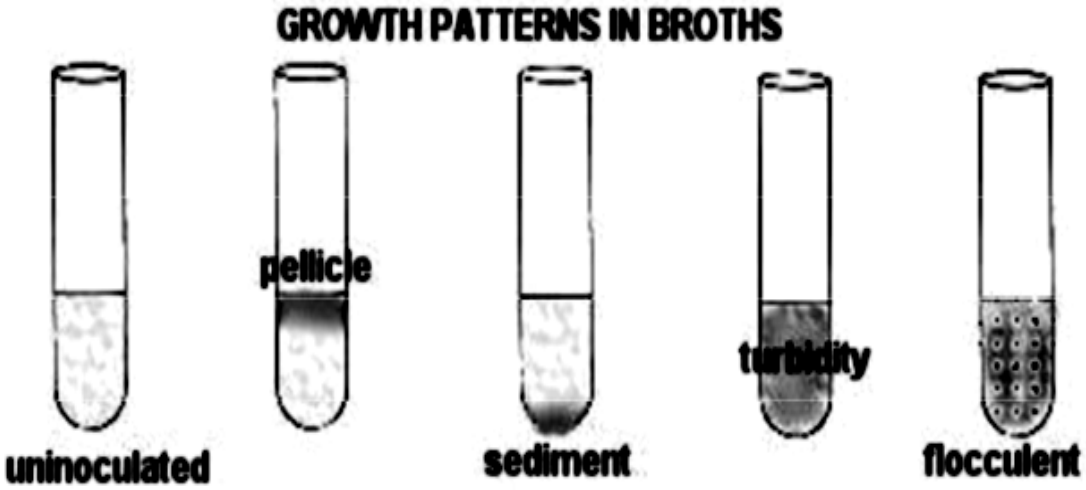
Shape	 Circular, Rhizoid, Irregular, Filamentous, Spindle
Margin	 Entire, Undulate, Lobate, Curled, Rhizoid, Filamentous
Elevation	 Flat, Raised, Convex, Pulvinate, Umbonate
Size	 Punctiform, Small, Moderate, Large
Texture	Smooth or rough ,**dry, moist, mucoid, rugose (wrinkled).**
Appearance	Glistening (shiny) or dull
Pigmentation	Nonpigmented (e.g., cream, tan, white) Pigmented (e.g., purple, red, yellow)
Optical property	Opaque, translucent, transparent

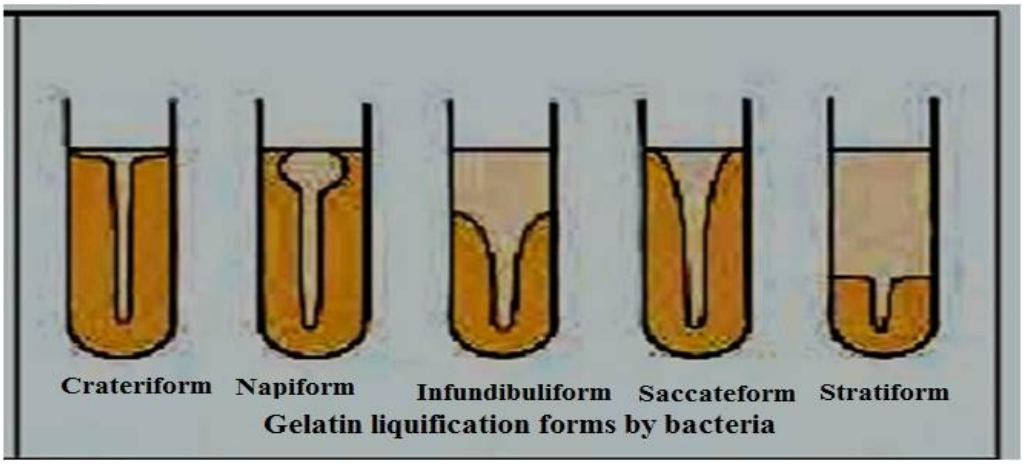


Bacterial growth pattern on Slant



Types of surface growth in nutrient broth





Gelatin liquification forms by bacteria

3. Identification of microorganisms on the basis of Biochemical characteristics-

Living organisms are differentiated on the basis of various enzyme-catalysed metabolic reactions, presence or absence of certain enzymes, intermediary metabolites, or end products often give valuable- information in identifying and classifying the organisms. A variety of biochemical tests, performed in well defined media and under standardized conditions of growth, arc available:

a. Fermentation and oxidation of carbohydrates.
b. Hydrolysis of starch and cellulose.
c. Hydrolysis and liquefaction of gelatin, coagulated serum, and casein.
d. Production of indole, hydrogen sulphide, acetyl methyl carbinol etc, in specific inoculated media, Reduction of nitrate, sulphate, methylene blue, or litmus in specific inoculated media.
e. Assay of specific enzymes such as catalase, indophenol oxidase, amino acid deaminase and decarboxylase, urease, cellulase, α & β amylases, phosphatase, coagulase, hyaluronidase, lecithinase, etc.

4. Identification of microorganisms on the basis of nutritional Requirements

All living organisms require water, a source of **energy, carbon, nitrogen, sulphur and phosphorus** in either inorganic or organic form. In addition to either small or trace amounts of several metallic elements, organisms also require vitamins or growth factors. **Autotrophs** are capable of synthesizing their entire cellular constituents from simple inorganic

compounds in the medium, but **heterotrophs** fail to grow unless one or more essential metabolites, growth factors, or vitamins are provided in the medium. The growth of strict autotrophs is inhibited in the presence of organic compounds; while facultative autotrophs grow equally well in the presence of either inorganic or organic compounds. Phototrophs obtain energy from the sun, where the growth is dependent upon exogenous inorganic hydrogen donors (photolithotrophs) or organic hydrogen donors (photoorgonotrophs}. Microorganisms can be divided into many nutritional groups on the basis of their nutritional requirements.

5. Identification of microorganisms on the basis of Physiological properties.

Physiological tests which are commonly used in the identification of an organism are as follows -

a. Temperature range of growth *(psychrophilic, mesophilic, thermophilic,* or *thermoduric).*
b. Oxygen tolerance *(strict aerobe, strict anaerobe, facultative anaerobe,* or *microaerophilic).*
c. pH range of growth *(acidophilic, neutrophilic,* or *alkaliphilic).*
d. Pigment production (water soluble or insoluble).
e. Tolerance and requirement of salt.
f. Source of illumination (photosynthetic organisms).

6. Identification of microorganisms on the basis of Serological properties

Microorganisms contain numerous antigenic substances which stimulate animals to produce antibodies. These antibodies react specifically with antigens. Antibodies are found in the sera of inoculated animals. The sera are then used to detect corresponding antigens. For example, a known culture is inoculated into an animal to stimulate antibody production. The serum of the inoculated animal is mixed with a suspension of the unknown microorganism. If there is a reaction (agglutination), then the unknown culture is either identical with the known culture, or at least the two organisms are very closely related in antigenic properties.

Serologic typing provides a means of identifying species and also reveals very subtle differences between individuals of a given species. For example, *Streptococcus pneumonias* fall into a number of distinct antigenic groups because of the differences in molecular structures of the capsular polysaccharides. The different strains are called types; Type I, Type II, etc. These types are solely

based on antigenic differences. These are often spoken of as *serotypes* because the antibodies used to determine the different types are found in the serum.

7. Identification of microorganisms on the basis of DNA base Composition –

Although DNA base composition may be determined chemically (hydrolyzing DNA sample and separating the free bases) it can be determined more easily by physical methods that are now mostly used. The melting temperature or buoyant density of DNA (i.e. the temperature at which it becomes denatured, by breakage of the hydrogen bonds holding the two strands together) is directly related to G+C content. Strand separation is accompanied by marked increase in absorbance at 260 nm, the absorption maximum of DNA. When a DNA sample is gradually heated, the absorbance increases as the hydrogen bonds are broken and reaches a plateau at a temperature at which the DNA has all become single stranded.

The midpoint of this rise, the melting temperature (Tm) is a measure of the G+C content. The G+C content may also be determined by subjecting a DNA sample to centrifugation in a CsCl gradient, which affords a precise measure of its density. This method can be used because the density of DNA is also a function of the (G+C): (A+ T) ratio.

DNA Base Composition of Representative Bacteria -

G+C (moles %)	**Organisms**
28-30	*Spirillum linum.*
30-32	*Clostridium perfringens. C. tetani*
32-34	*C. bitermentans. Leptospira pomona. Staphylococcus aureus*
34-36	*Bacillus anthracis, Mycoplasma gallisepticum, Staphylococcus albus, streptococcus faecalis, Treponema pallidium*
38-40	*Bacillus megaterium. Hemophilus influenzae, Streptococcus pneumoniae. Proteus vulgaris*
40-42	*Bacillus laterosporus, Leptospira bifexa, Neisseria catarrhalis*
42-44	*Bacillus subtilis, B. stearothermophilus*
46-48	*Clostridium nigrificans, Corynebacterium acnes, Vibrio cholerae*
48-50	*Neisseria gonorrhoeae*

50-52	*Bacillus macerans, Escherichia coli, Salmonella spp, Shigella spp.*
52-54	*Enterobacter spp., Corynebacterium diphtheriae*
54-56	*Alcaligenes faecalis. Azotobacter agile. Brucella abortus*
56-58	*Corynebacterium spp., Lactobacillus bitidus*
58-60	*Agrobacterium tumefaciens, Corynebacterium spp., Serratia marcescens*
60-62	*Azotobacter vinlandii, Rhodospirillum rubrum, Vibrio spp.*
64-66	*Desulfovibrio desulfuricans, Pseudomonas spp.*
66-68	*Pseudomonas aeruginosa, Mycobacterium tuberculosis*
68-70	*Pseudomonas saccharophila, Sarcina flava*
70-80	*Mycobacterium smegmatis, Sarcina lutea,*

Constancy of G+C Content in Strains Of Bacteria -

Pseudomonas spp	**No. of strains examined**	**G+C content of DNA, mole per cent (mean value + - standard deviation)**
P. aeruginosa	11	67.2+ - 1.1
P. acidovorans	15	66.8+ - 1.0
P. testosteroni	9	61.8+ - 1.0
P. cepacia	12	67.6+ - 0.8
P.pseudomallei	6	69.5+ - 0.7
P. putida	6	62.5+ - 0.9

8) Identification of microorganisms on the basis of Nucleic Acid Hybridization

(DNA-DNA homology or DNA base sequence and RNA sequences).

Similarity of base composition represents only a limited basis for close genetic relatedness because even distant organisms can by chance have a similar composition. Moreover as groups diverge in evolution the DNA sequence changes long before the base composition. Homology of sequence (DNA - DNA homology) can be measured quantitatively in terms of the ability of DNA strands from two different sources to form **molecular**

hybrids in vitro. Surveys among higher organisms have revealed close parallelism between the results of such hybridization and the phylogenetic relations concluded on other grounds.

Surveys among higher organisms have revealed close parallelism between the results of such hybridization and the phylogenetic relations concluded on other grounds. When DNA preparations from two related strains of bacteria were mixed and treated at 10 to 30°C below the Tm value. Hybrid DNA molecules are formed. However DNA preparations from two unrelated bacteria could not hybridize.

This discovery of re-association of single stranded DNA molecules (single strands formed due to treatment given at a temperature. below Tm value) from different biological sources to form hybrid duplexes laid the foundations of DNA-DNA homology in bacterial genetic relatedness. Among bacteria this is useful only within closely related groups because it quickly disappears in the wider range of variation. Since duplexes can also be formed between single-stranded DNA and complementary RNA strands analogous DNA-RNA re-associations can be performed. Ribosomal RNA hybridization to DNA is useful for estimating more distant kinship among bacteria.

 RNA sequences have proved more useful than DNA sequences because they involve relatively small segments of the chromosome: those that code the base sequence either of the tRNAs or of the rRNAs. An alternative is thus to sequence the smaller molecules (RNA or protein) derived from DNA. This technique is thus used for comparative sequencing of specific RNA molecules.

 The molecules are highly conserved in an evolutionary sense and provide a very useful measure of the phylogenetic relationships of the wider groupings of microorganisms. The chosen molecules are ribosomal RNA components, the 16S rRNA and the 5S rRNA. However, nucleic acid hybridizations have not yet provided a phylogenetic bacterial taxonomy to replace the classical Bergey's Determinative key.

9) Identification of microorganisms based on 16s rDNA sequence analysis

INTRODUCTION:

The rDNA is the most conserved (least variable) gene in all cells which encode rRNA. Portions of the rDNA sequence from distantly-related organisms are remarkably similar. This means that sequences from distantly related organisms can be precisely aligned, making the true differences easy to measure. For this reason, rDNA genes that encode the rRNA have been used extensively to determine **taxonomy**, **phylogeny** (evolutionary relationships), and to estimate **rates of species divergence** among bacteria. Thus the comparison of 16s rDNA sequence can show evolutionary relatedness among microorganisms. This work was pioneered by Carl Woese, who proposed the **three Domain system** of classification - Archaea, Bacteria, and Eucarya - based on such sequence information.

The Ribosomal RNAs

In Bacteria, Archaea, Mitochondria, and Chloroplasts the small ribosomal subunit contains the 16S rRNA (where the S in 16S represents Svedberg units). The large ribosomal subunit contains two rRNA species (the 5S and 23S rRNAs). Bacterial 16S, 23S, and 5S rRNA genes are typically organized as a co-transcribed **operon**. There may be one or more copies of the operon dispersed in the genome (for example, *E coli* has seven). The Archaea contains either a single rDNA operon or multiple copies of the operon.

To infer relationships that span the diversity of known life, it is necessary to look at genes conserved through the billions of years of evolutionary divergence. An example of genes in this category is those that define the ribosomal RNAs (rRNAs). Most prokaryotes have three rRNAs, called the 5S, 16S and 23S rRNA.

Ribosomal RNAs in Prokaryotes

Name	Size (nucleotides)	Location
5S	120	Large subunit of ribosome
16S	1500	Small subunit of ribosome
23S	2900	Large subunit of ribosome

The 5S has been extensively studied, but it is usually too small for reliable phylogenetic inference. The 16S and 23S rRNAs are sufficiently large to be useful. The 16s rDNA sequence has **hypervariable regions**, where sequences have diverged over evolutionary time. These are often flanked by strongly-conserved regions. Primers are designed to bind to **conserved** regions and amplify variable regions. The DNA sequence of the16S rDNA gene has been determined for an extremely large number of species. In fact, there is no other gene that has been as well characterized in as many species. Sequences from tens of thousands of clinical and environmental isolates are available over the internet through the **National Center for Biotechnology Information** (www.ncbi.nlm.nih.gov) and the **Ribosomal Database Project** (www.cme.msu.edu/RDP/html/index.html). These sites also provide search algorithms to compare new sequences to their database.

16sSequencing

The extraordinary conservation of rRNA genes can be seen in these fragments of the small subunit (16S) rRNA gene sequences from organisms spanning the known diversity of life. Note several areas of identity among these diverse organisms:

Human

GTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCA
GTTAAAAAG

Yeast

GTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGC
AGTTAAAAAG

Corn

GTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCA
GTTAAAAAG

Escherichia coli

GTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGG
GCGTAAAGCG

If relationships were analyzed by comparing sequence data, rather than hybridizing the molecules, one could infer relationships without having all of the molecules in hand (only the sequence data from previous studies are necessary). This was already being done with protein sequences.

Carl Woese recognized the full potential of rRNA sequences as a measure of phylogenetic relatedness. He initially used a RNA sequencing method that determined about 1/4 of the nucleotides in the 16S rRNA (the best technology available at the time). This amount of data greatly exceeded anything else then available. Using newer methods, it is now routine to determine the sequence of the entire 16S rRNA molecule. Today, the accumulated 16S rRNA sequences (about 10,000) constitute the largest body of data available for inferring relationships among organisms.

Molecular Phylogenies can reflect genealogy and amount of change by comparing the inferred rRNA sequences (or those of any other appropriate molecule) it is possible to estimate the historical branching order of the species, and also the total amount of sequence change. An example of a 16S rRNA-based **phylogenetic tree** showing the three (identified) Domains of life - **Bacteria, Archaea** and **Eucarya.**

PROCEDURE:

In this laboratory, amplify a region of the 16s rDNA gene (based on the *E. coli* 16s rDNA sequence) using Polymerase Chain Reaction (PCR). The DNA will be sequenced using the capillary DNA sequencer at the Grice Marine Lab. We will then compare the results obtained with the public databases (NCBI and RDP) and determine the identity of the unknown bacteria.

❖ Classification of Bacteria on the basis of (Bergey's Manual of Determinative Bacteriology)

The artificial scheme of classification in *Bergey's Manual of Systematic Bacteriology* is widely used. *Bergey's Manual* disregards evolutionary relationships because they often group bacteria into assemblages that cannot be easily identified by standard laboratory procedures. Instead, the manual takes a strictly practical approach so that it can be used as a comprehensive & quick reference when accuracy & speed are important, as is often the case in diagnostic labs.

Bergey's Manual divides bacteria into 4 divisions on the basis of their cell wall [G(+) or G(-)], their lack of a cell wall (mycoplasmas), & walls lacking peptidoglycan (archaeobacteria). Bacteria species in each division are assigned to one or two sections; sections have no taxonomic standing; they are simply groups of bacteria, which share certain easily identifiable properties.

Classification of Bacteria from Bergey's Manual of Determinative Bacteriology -

Parts	**Kingdom - Procaryotae Division - Bacteria**	
Part 1	Phototrophic bacteria	Aquatic bacteria that produce carbohydrates from CO_2 using photosynthetic pigments.
Part 2	Gliding bacteria	Rods that move by gliding in a layer of slime; many form complex fruiting bodies.
Part 3	Sheathed bacteria	Rods surrounded by a sheath of iron or manganese oxides; some have flagella.
Part 4	Budding and/or appendaged bacteria	Diverse group of rods that reproduce by forming buds or appendages,
Part 5	Spirochetes	Slender, helically-coiled bacteria moving by rotation or flexion of the cell.
Part 6	Spiral and curved bacteria	Helically-curved rods that move with a cork-screw-like motion.
Part 7	Gram-negative aerobic rods and cocci	Rods and spheres that give a negative reaction on Gram staining and that require oxygen for life.
Part 8	Gram-negative facultatively anaerobic rods	Rods that give a negative reaction on Gram staining and that may survive in the absence of oxygen
Part 9	Gram-negative anaerobic rods	Rods that give a negative reaction on Gram staining and that require an oxygen-free environment for life
Part 10	Gram-negative cocci and -coccobacilli	Spheres that give a negative reaction on Gram staining.
Part 11	Gram-negative anaerobic	Spheres that give a negative

	cocci	reaction on Gram staining and that require an oxygen-free environment for life,
Part 12	Chemolithotrophic bacteria	Bacteria that use nitrogen, sulfur, and iron compounds for energy and structural components
Part 13	Methane-producing bacteria.	Rods and spheres that obtain energy from carbohydrates and that form methane as an end product
Part 14	Gram-positive cocci	Spheres that live in the presence or absence of oxygen and that give a positive reaction no Gram staining
Part 15	Endospore-forming rods and cocci	Rods and spheres that form endospores during their life cycles.
Part 16	Gram-positive as porogenous rods	Rods that do not form spores and that give a porogenous rods positive reaction on Gram staining.
Part 17	Actinomycetes and related organisms	Large group of aerobic and anaerobic rods including many soil forms.
Part 18	The rickettsias	Small rod-shaped bacteria that are transmitted by arthropods and that multiply only within a host cell; includes chlamydiae,
Part 19	The mycoplasmas	Very small, multi-shaped bacteria lacking a true cell wall

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