MICROBIOLOGY

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Contents

1. Introduction

Microbiology is the study of microscopic organisms. These are very ancient organisms originating about four billion years ago (*see Biological Science Foundations*). However, the study of these organisms started only three hundred years ago with the invention of the microscope. The importance of microorganisms became apparent in the 19th century when it was shown that they were central to many fundamental processes in life ranging from the production of beer and wine to the causation of disease. The enormous diversity of microscopic life which is, even today, not really understood means that they are capable of carrying almost any chemical task that is put to them.

The simplicity of microorganisms makes them a very important tool in the scientific analysis of life processes. Most of the essential biochemical processes found in higher organisms have the counterpart in micro-organisms and in many cases they biochemical process is essentially the same. As has been discussed in (*see DNA as the Genetic*

Material and Nucleic Acid Metabolism), the genetic information in all cellular organisms is DNA. Because they are relatively simple organisms which have a rapid rate of reproduction coupled with a wide range of biochemical activities, they have because the first choice in many investigations of basic biological processes.

They are also a source of products for society. They have been used for many years to produce some products, for example, alcohol by yeast, and have been used in the twentieth century to produce new products such as acetone/butanol by *Clostridium acetobutyricum* and single cell protein from hydrocarbon wastes by a variety of microorganisms. These processes, and many others, form what is called biotechnology or the exploitation of biological system, particularly not exclusively microbiological systems for economic benefit. The advent of genetically engineered microorganisms has vastly increased the potential for microbial biotechnology and it is now possible to produce, for example, human insulin from microorganisms. It is no longer necessary to extract bovine or porcine insulin from animal pancreases in order to treat diabetes.

The scope of microbiology is very large. Scientists have probably, at best, only investigated 0.1% of all types of microorganisms found on earth and possibly a lot less than that percentage. Microorganisms can be found in almost any environment on earth where there is water and can survive for many years even in the absence of water. Look around you, you do not see them but your environment is filled with millions of different microorganisms. Studying them is Microbiology.

2. Taxonomy

A large part of microbiology in the past has been concerned with describing and classifying microorganisms. By so doing, the microbiologist was able to identify specific microorganisms when found at a future time. He could also relate a specific organism to a specific process such as food spoilage or a human disease. Finally, he can try and identify similarities and differences between particular microorganisms and attempt to build up a family tree of microorganisms that relates to their evolution over the last four billion years.

The methods used to classify microorganisms have improved with the general developments in biological science and mathematics. The latter is important as the large number of organisms under study and the large number of characters measured mean that statistically sound mathematical analysis is needed to be able to draw meaningful conclusions and identifications. Initially, in the 19th century, morphology was the method used to classify microorganisms. During the 20th century, the growth characteristics and enzyme activities of the microorganisms became important as classification tools. As molecular genetics came to the fore during the latter half of the 20th century, molecular approaches to taxonomy became important. As we enter the 21st century, as more and more complete genomes of microorganisms become available, the genomic approach to taxonomy and evolution of microorganisms becomes possible.

All the methods described above relate directly to the genetic material. Morphology is merely as measure of the expression of a specific group of genes involved in cell structure. Growth characteristics reflect genes involved in metabolism. Molecular genetics also

reflects specific groups of genes identified as molecular marker of taxonomy such as 16S ribosomal RNA genes. None characterize completely a particular organism but give a snapshot of that organism through those genes. All are important and genomics begins to bring the complete organism into the picture.

2.1 Morphology

It is important to note that morphology remains a cornerstone of microbiological identification. Initially, it allows the separation of microorganism into eucaryotes and prokaryotes on the basis of the presence in the former of intracellular structures (Figure 1). The presence of a nucleus, a mitochondrion or a chloroplast is diagnostic of the organism being a eukaryote. The fact that an organism is multicellular is not diagnostic as the long time positioning of the *Streptomyces*, a multicellular prokaryote, in the eukaryote fungal group testifies to. Only when *Streptomyces* became important for their antibiotic production was their small cellular size and lack of intracellular structures used to reclassify them correctly.

2.1.1 Eucaryote Microorganisms - Protozoa

These are single-celled eucaryotic microorganisms that can ingest found from their growth medium, lack a rigid cell wall and do not contain chloroplasts. Many are mobile using *cilia* or *flagella* to move themselves through the liquid they are found in. Some do not swim but move by cellular protrusion or *pseudopodium* and are called *amoeba*. Some can form resting spores to survive environmental changes. They occur widely in nature particularly in aquatic environments and some can cause diseases, and example being malaria.

2.1.2 Eucaryote Microorganisms - Algae

Algae are eucaryotic single cell or multicellular microorganisms that contain chloroplasts and have rigid cell walls. They vary greatly in size and some can be up to several meters in length and not really microorganisms at all. They obtain their energy for growth from sunlight although many can utilize nutrients in their growth media to growth in the absence of light. They also can be motile and usually use *flagella* for movement.

2.1.3 Eucaryotic Microorganisms - Fungi

Fungi are eucaryotic single or multicellular microorganism with a rigid cell wall but they do not contain chloroplasts. They also vary greatly in size and some can be up to almost a meter in size such as the bracket fungi that growth on trees. Fungi do not ingest food but absorb it from the environment usually after releasing enzymes to help break down their environment. Unicellular fungi are called yeasts while multicellular fungi are molds or mushrooms. Most fungi can produce spores as part of their reproduction and spore production bodies form an important part of their morphological classification. A wide range of biotechnological processes use fungi such as cheese making, they are important as degraders of organic material and so can be pathogenic.

2.1.4 Procaryotic Microorganisms – Bacteria and Archaea

The prokaryotes or bacteria are divided into two Kingdoms, the Bacteria and the Archaea. They are distinguished both on molecular genetic grounds and because have distinctly different structural features (Table 1). There are no known diseases caused by members of the Archaea and they exist mainly in extreme environments such a hot springs, deep-sea hydrothermal vents and in saltpans. The major morphological division of the bacteria has been based on their cell wall structure. With a specific chemical staining technique developed by Gram, the so-called Gram stain, certain bacteria absorb the purple stain onto the cell wall and are called Gram $+^{ve}$ while others do not and are called Gram -ve. This division remains very important as it reflects a fundamental biochemical difference in the evolution of these groups of bacteria. This is true for the Eubacteria, however, the Archaea, an even more fundamental grouping identified by molecular techniques, cannot be classified this way.

	Bacteria	**Archaea**	**Eukarya**
Cell type	Prokaryotic.	Prokaryotic.	Eukaryotic.
Typical size	0.5μm to 4.0μm, but can be larger.	0.5μm to 4.0μm.	Greater 5μm.
Cell wall	Usually present and Contains peptidoglycan.	Usually present and lacks peptidoglycan.	Usually absent.
Cell membrane	Fatty acids present with ester linkages.	Isoprenes present with ester linkages.	Fatty acids present with ester linkages.
Protein synthesis	Start amino acid is formlymethionine. Inhibited by antibiotics such as chloramphenicol.	Start amino acid is methionine. Not inhibited by antibiotics such as chloramphenicol.	Start amino acid is methionine. Not inhibited by antibiotics such as chloramphenicol.
Genetic material	Mostly small circular chromosomes, but some linear chromosomes. No histones bound to DNA.	Small circular chromosomes. Histone-like proteins Bound to DNA.	Complex nucleus. Large linear chromosomes. Histones bound to DNA.
Organelles	No membrane bound organelles.	No membrane bound organelles.	Membrane bound organelles containing DNA.
RNA polymerase	Simple.	Complex.	Complex.
Habitat	Wide range.	Extreme.	Wide range.

Table 1: Comparison of Bacteria, Archaea and Eukarya

The other major morphological characteristics of bacteria are:

Shape: Sphere like or cocci; Rod like or bacilli; Spiral shaped or spirilla

Size: This ranges usually from 0.5 to 5.0 um

Clustering: Singly, in pairs, in tetrads, in chains, in clusters or as mycelium

Spore formations: If spores are formed and, if so, their position in the cell.

Chlorophyll: Certain bacteria are capable of photosynthesis using pigments directly in their membrane

2.1.5 Viruses

Viruses are not living organisms in themselves and can only reproduce with the help of a cellular organism. They do not have a cell structure and are much smaller than even bacteria, usually between 20 and 300 nm in size. Morphologically, they consist of nucleic acid surrounded by a protein coat. The shape of the virus can be observed under the electron microscope and can range from filaments to very specific geometric patterns of protein.

2.2 Chemical Taxonomy

Once it became possible to cultivate microorganisms in pure culture on Petri dishes using agar as a growth medium (4.0), it was noticed that if chemically defined media was used, bacteria could be classified on their ability to grow or not grow on certain media. This growth effectively was dependent on the presence or absence of certain enzymes necessary for the utilization of the chemicals present in the defined medium. Thus it was possible to design chemical tests for their utilization. As the presence or absence of the enzyme is genetic in basis, these tests effectively were a method of establishing, in a limited way, the genetic status of a particular microorganism. Using a wide battery of such tests, it proved possible to classify and identify many bacteria in this way. The method is speedy and does not require complex equipment and is widely used for these purposes.

It's limitations are three fold: firstly, there are many bacteria that either do not grow at all on plates or do not grow on defined chemical media and these cannot be classify in this manner; secondly, while an individual bacterial clone may have a specific chemical taxonomy profile, bacteria of the same species may differ to some extent even though they are effectively the same species and thus some isolates just do not fit the system; finally, the broad range of the test makes the need to computerize the system necessary with different testing systems requiring different databases leading to lack of compatibility between systems. But overall, it has been and still is a very useful system of classification and has been successfully used for taxonomic purposes, so called numerical taxonomy.

In theory all microorganism capable of growth in chemically defined media should be able to classified using chemical taxonomy. The system has been widely developed for the following organisms with tests that are really not cross compatible between groups of organisms. These are Gram +ve bacteria, Gram -ve bacteria, yeasts and some other fungi.

2.3 Molecular Taxonomy

The ability to access individual genes as units of taxonomy created a revolution in the study of the relationships between microorganism as well as the evolution of microorganisms. It allowed a great deal more information to be processed during the analysis and therefore, in theory, increased the accuracy of the results produced. However, it also produced problems of disagreements between different approaches as well as questions as to the best way to analyze such large data sets.

2.3.1 Protein based taxonomy

Within each cell of a unicellular microorganism, are the total complements of proteins being produced by that organism from its genes, under the physiological conditions prevailing at that time. It is slightly different for multicellular microorganisms where tissue differentiation can result in different tissues expressing different proteins and thus this must be taken into account with these. An analysis of the proteins being produced under a particular set of physiological conditions is thus a reflection of the genetic makeup of that organism. Different organisms will produce different ranges of proteins under the same condition. The same protein produced by different organisms could different in molecular weight and/or charge as well as biochemical activity. A comparison of the proteins produced by microorganisms under the same conditions therefore allows the scientist to measure the similarities and differences between these organisms whether they are species or strains. Obviously, these similarities and differences can be used to estimate the relationships at a gene level of the organisms under study

The technology involved in this approach divides the data produced into two types. The first compares only the molecular weights of the proteins from particular organisms, while the second compares the charge, molecular size and activity of the proteins from particular organisms. The first technique uses SDS to equalize the charge on all the proteins but in the process generally inactivates them. The second uses native proteins but requires that specific activity dyes or antibodies are used to determine the presence or absence of a particular enzyme or protein. Both systems use electrophoresis to separate the proteins usually on a gel matrix

The first method or SDS PAGE of proteins produces a dataset that includes the molecular weights of the proteins detected (usually by a general protein stain) against molecular weight standards. The presence or absence of a protein band at a molecular weight can be compared between strains and used to calculated similarity. It should be noted that this system does not guarantee that a protein at a particular molecular weight is the same protein in two strains. They may not be and this creates a degree of noise within the system. Equally, the detection system may limit the ability of the system to detect low quantities of a protein and this can also create noise. In general, it is not usual to include the amount of protein detected in analyze of this type of data as again this can lead to increased noise under circumstances. This technique is a simple easy method of strain comparison but is not that useful as larger evolutionary distances.

The second method or activity gel analysis also involves the separation of proteins, this time usually on a starch gel matrix. Proteins are separated by electrophoresis from the centre of the gel towards both the positive and negative electrodes at a rate dependent on

their charge. They are also separated on the gel by molecular weight. Once electrophoresis is complete, the gel is stained for the presence of a particular enzyme and the position of the band(s) for this enzyme type noted. This is obviously more complex than method described above but collects more information on each particular enzyme analyzed. Finally, a similar dot matrix is produced and compared for the strains under study. Direct comparison is possible between the particular bands noted and therefore the system is less open to noise. However, it is more costly and more time consuming. A variation between the two systems can also be used whereby proteins are separated by SDS PAGE but detected by antibodies specific to particular proteins.

In general, the methods described above are useful for analysis between closely related species but break down if pushed beyond this because they only indirectly look at the genetic material itself.

2.3.2 DNA based taxonomy

Because of the inherent limitations of the methods described in 2.3.1, because advancing techniques began to allow scientists to look directly at the genetic material, it was obvious that this approach to taxonomic and evolutionary studies provides a number of advantages. These are: firstly, the genetic material is unaffected by the physiological state of the organism; secondly, the information at the level of DNA is much more detailed that at any other level; and finally, the information needed could be targeted from particular parts of the whole genetic makeup of the organism. The first two advantages remain valid although the last is now subject to reinterpretation as we shall see later.

DNA based taxonomy can really be divided into two major categories which are then subject to further subdivision. These are DNA fragment based analysis and DNA sequence based analysis. DNA fragment based analysis can be subdivided into gene specific fragment analysis and random fragment analysis while DNA sequence based analysis is subdivided based on the gene sequence analyzed such as the gene(s) for the ribosomal RNA, heat shock protein genes, *tufA* gene, *etc.*

The technology for gene specific fragment analysis varies but the system is based on the detection of specific gene fragments cut with a variety of restriction endonucleases. Restriction endonucleases are enzymes that cut DNA at a specific palandromic base sequence which varies from four to eight base pairs in length usually. The presence of such a sequence in a given gene is dependent on the sequence of the gene and changes in the sequences between different organisms will be reflected in changes in the presence of the cutting sites. This, in turn, will change the size of the fragments produced. These changes in fragment size can be used to calculate the similarities/differences between the genes in the different organisms. The choice of gene and the method of specifically detecting the gene fragments are the major variables in this system.

Random fragment analysis differs from the above system in that it is generally polymerase chain reaction based and used short usually ten base pair single primers to amplify fragments that match these primers from the total genome of the organism. This so-called randomly amplified polymorphic DNA polymerase chain reaction or RAPD-PCR creates a series of DNA fragments usually varying from 200 base pairs to

5000 base pairs. The fragments form a selection from the genome under study and are not specific to any particular gene and therefore unlike the above system or representative of the complete genome of the organism. Identical organisms under identical conditions give the same pattern of fragments. Evolutionary changes in the base sequence of the organism will change the priming sites of the primer and either remove a band or add a band. Overall genome similarity between different organisms can therefore be calculated. RAPD-PCR's limitation is that, while reproducible within a single laboratory using a single thermal cycler for the PCR, it is difficult to transfer data between laboratories. However, it is a technique which avoids some of the problems associated with single gene methods as described in 2.3.4.

DNA based sequence analysis is now widely used for comparison of different organisms. However, it is generally based on choosing a particular gene for the analysis. The choice of the gene can be very important. In microbiology, the gene usually chosen is the ribosomal RNA gene either the 16S in prokaryotes or the 18S in eucaryotes (2.3.3). However, other genes can be used and a trend towards multiple gene analysis for taxonomic purposes is now apparent.

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Biographical Sketch

Ralph Kirby is Professor of Microbiology at Rhodes University, Grahamstown, South Africa. He has held this position for the last ten years. He graduated with his B.A. at Trinity College, Cambridge, UK, in 1972, and completed his Ph.D. at the University of East Anglia, UK, in 1976. Post-doctoral research followed at the University of Bristol, UK, then a lectureship and senior lectureship at the University of Cape Town, South Africa. His major interests are the molecular genetics of Actinomycetes, horizontal gene transfer, molecular population genetics, and the interaction between law and science. He is presently completing a LL.B.

Timeline

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