CHAPTER-1

Introduction and Historical Perspective

Soil Microbiology is a branch of soil science concerned with microorganisms found in the soil and their relationship to soil management, agricultural production, and environmental quality. Hence, the soil microbiologist studies the numbers and kinds of microorganisms found in soil and the effect of these and introduced microorganisms on soil-ecological processes (e.g., nutrient cycling). The applications of these studies have important consequences for crop production, environmental quality, and the restoration of compromised environments.

The soil is a complex habitat for microbial growth. It differs markedly from the environment microorganisms encounter in traditional microbiological culture media in two crucial ways:

- In its natural state, the soil is a heterogeneous medium of solid, liquid, and gaseous phases, varying in its properties, both across the landscape and in depth.
- In soil, competition exists among a wide variety of organisms for nutrients, space, and moisture. Competition occurs among bacteria, actinomycetes, and fungi, as well as with other living forms in soil, including animals and plant roots.

If we are to understand soil microorganisms, then developing a knowledge of the habitat in which they grow is of utmost importance.

The Nature of Cellular Organisms

The basis of living matter is the cell. Each cell is a unique entity made up of a complex mixture of chemical materials and subcellular components. The cell is bounded by the cytoplasmic membrane**,** separating the interior of the cell, known as the cytoplasm, from the external environment.

Two fundamental types of living cells are recognized: *prokaryote* (from *pro*, meaning "before," and karryon, meaning "nucleus") and *eukaryote* (from *eu*, meaning "true"). Major structural differences exist between the two types of cells. The nucleus of the eukaryote is in the cytoplasm, bounded by a nuclear membrane and containing several DNA molecules. The eukaryote undergoes division by the well-known process of mitosis. The prokaryote has no nucleus; a nuclear region is recognized, but it is not bounded by a membrane and consists of a single, circular DNA molecule (chromosome). Cell division in the prokaryote is usually by binary division (i.e., nonmitotic). Additional differences between prokaryotic and eukaryotic cells are presented in Table-1. Bacteria (including cyanobacteria and actinomycetes) and Archaea are prokaryotes, while all other organisms are eukaryotes.

Characteristics of Living Cells. Five key characteristics have been recognized that separate living cells from nonliving chemical systems:

- *Self-feeding or nutrition*: The capacity to take up and use chemicals from the environment and transform these chemicals into usable products, including energy to grow or survive.
- *Self-replicating or growth:* The capacity to self-direct synthesis, growing by division, forming two cells from one.
- *Differentiation:* The capacity to undergo change in form or function, often in response to environmental changes or normal growth processes.
- *Chemical signaling*: The capacity to interact with other cells through chemical signals.
- *Evolution*: The capacity to change genetically, which may affect the overall fitness of the cell to survive in a particular environment.

Table 1: A structural comparison of prokaryotic and eukaryotic cells.

Organelle	Prokaryotes	Eukaryotes
Cytoplasmic membrane	+	+
Nuclear division	+	+
Nuclear membrane	-	+
Ribosomes	70S	80S
Endoplasmic reticulum	-	+
Golgi complexes	-	+
Mitochondria	-	+
Cytosketeton	-	+
Chloroplasts	-	- (+ for algae)
Vacuole	-	+
Cell wall	+	+

Classification of Organisms

The study and use of microorganisms is based on our ability to recognize and establish the identity of individuals. Most classification schemes are organized to show relationship among organisms. This orderly arrangement allows us to communicate descriptive information about the organism to others. These data can also be entered into various microbial databases, allowing retrieval of information about related organisms.

Microbiologists use the Linnean system of bionomial nomenclature to name the microorganisms with which they work. An organism's name is made up of genus and species. In higher organisms, *species* are defined as groups of interbreeding or potentially interbreeding natural populations; however, many microorganisms do not reproduce sexually so this definition is not very useful. Microbiologists define ***species*** as a group of similar individuals that are sufficiently different from other individuals to be considered a recognized taxonomic group. A collection of species that share a major property (or properties), making them a distinct grouping, permits the group to be considered a *genus* (plural, *genera*). Hence, the Latin binomial name, *Thiobacillus thiooxidans* (abbreviated *T. thiooxidans* after it is used the first time in the text) is representative of a group of individuals (species: *thiooxidans)* that have the capacity to oxidize sulfur and share some common characteristics with other organisms in the genus *Thiobacillus*. Often microorganisms are named for an outstanding feature they possess (e.g., *T. thiooxidans*; a rod-shaped bacterium capable of oxidizing reduced sulfur for the generation of energy). In other cases organisms are named to commemorate the contribution of an outstanding scientist in the field (e.g., *Nitrobacter winogradskyi*, named in the honor of the Russian soil microbiologist Sergei Winogradsky).

Historically, microorganisms were classified on the basis of taxonomic features, which were relatively easy to measure. These characters include structure, morphology, staining reactions, and physiological parameters (e.g., ability to use a particular carbohydrate). However, these features are phenotypic (based on physical characteristics) rather than phylogenetic (based on genetic relationships) and may obscure important relationships among related groups of organisms.

The technology of molecular sequencing has introduced a totally new way of determining relationships among organisms. Phylogenetic "trees," showing relationships among organisms, are constructed directly from comparisons of informational macromolecules, such as ribosomal RNA (rRNA) genes, occurring in living cells. The traditional classification scheme recognized five kingdoms of organisms: bacteria, fungi, protista (including algae and protozoa), animals and plants. However, molecular phylogeny shows that there are three domains of living organisms: Bacteria, Archaea and Eucarya. Although the placement of organisms into three domains was defined by differences within the rRNA gene, subsequent studies reveal that organisms in these domains also differ in cell wall properties, lipid composition, and protein synthesis. Below the three domains, eight or more kingdoms have been recognized.

In these classification schemes there is no place for viruses. Viruses are not cells because they lack a cytoplasmic membrane with internal cytoplasm. Only when viruses are associated with another organisms (e.g., bacterium, plant, animal) are they able to fulfill the basic life processes.

Organisms in the Soil

Organisms in the soil are both numerous and diverse. Many soil organisms are small and cannot be seen without the aid of magnification (Table 1-2). The smallest organisms-bacteria, actinomycetes, fungi, and algae-are referred to collectively as the **microflora.** Soil animals range in size from microscopic (**microfauna**) to earthworms and small mammals (**macrofauna**). With the exception of some soil animals and fungi, most soil organisms are single cells. For convenience, soil microbiologists express the numbers of microorganisms in soil in an exponential manner, a convention known as scientific notation. Two million organisms per gram of soil is written, 2×10^6 g^{-1}. The same number can also be expressed as a logarithmic number, $\log_{10} 6.31$ g^{-1}.

Bacteria are the most abundant microorganisms in soil, attaining populations in excess of one hundred million (10^8) individuals per gram (g^{-1}) of soil and representing perhaps as many as 10^4 to 10^6 different species. The actinomycetes and fungi are the next most numerous microorganisms in soil, numbering 10^6 to 10^7 and 10^4 to 10^6 g^{-1} soil, respectively. Numbers of soil animals vary widely in the soil, ranging from just a few to as many as 10^6 g^{-1} soil. It is important to note, however, that we must consider more than the number of individuals in a gram of soil if we are to understand microbial function in soil. Microorganisms have a wide range of size and morphologies; thus, numbers alone may not provide a very good indication of the importance of a microbial group in the soil. For example, even though bacterial numbers are usually several orders of magnitude greater than fungi, the fungi generally have a greater total biomass in the soils (Table 2).

The physical, chemical, and even biological properties of the soil habitat and their interaction with the resident community of soil microorganisms have a significant impact on growth and activity of microorganisms. As our understanding of these complex relationships develops, we should be better able to manage the soil and its microorganisms for the maintenance and improvement of soil without damaging the soil as a resource.

Table 2: Microbial groups with representative size, numbers, and biomass found in soil.

Microbial group	**Example**	**Size (µm)**	**Numbers (no. g^{-1} soil)**	**Biomass (kg wet mass ha^{-1} soil)**
Viruses	Tobacco mosaic	0.02 ×0.3	10^{10} - 10^{11}	
Bacteria	*Pseudomonas*	0.5 × 1.5	10^{8}– 10^{9}	300-3,000
Actinomycetes	*Streptomyces*	0.5 – 2.0	10^{7} - 10^{8}	300- 3,000
Fungi	*Mucor*	8.0	10^{5}- 10^{6}	500- 5,000
Algae	*Chlorella*	5 × 13	10^{3} – 10^{6}	10 – 1,500
Protozoa	*Euglena*	15 × 50	10^{3}- 10^{5}	5- 200
Nematodes	*Pratylenchus*	1,000*	10^{1} - 10^{2}	1- 100
Earthworms	*Lumbricus*	100,000*		10- 1,000

*length

Microorganisms have an enormous diversity of functions in the soil. For example microorganisms decompose organic compounds releasing inorganic elements, oxidize reduced forms of elements (e.g., elemental sulfur $\rightarrow$ sulfate) and reduce oxidized forms of elements (e.g., nitrate $\rightarrow$ dinitrogen). Also, the reduction of dinitrogen to a biologically usable form, ammonia, or degradation of organic wastes and pollutants to carbon dioxide and water are important functions of soil microorganisms. Interactions among different organisms, including plant roots, can lead to benefits for some participating organisms, while others have a detrimental effect on growth or development. Other microorganisms may alter levels of global gases.

The Historical Context of Soil Microbiology

The development of soil microbiology is linked with the development of microbiology as a science and cannot be understood in isolation. Here we highlight significant accomplishments that led to the development of both microbiology and soil microbiology (Table 3).

Pre-Nineteenth Century

The first historical mention of the presence of microscopic organisms in soil is attributed to a Roman writer in about 60 B.C. Writing about marshes, Columella noted they gave up “noxious and poisonous steams,” breeding “animals armed with poisonous stings,” from which “hidden diseases are often contracted, the causes of which even physicians cannot properly understand.” Even prior to that time, there are reports in the Old Testament of people who practiced strict isolation and cleanliness codes, particularly to those afflicted with leprosy (a chronic disease caused by infection with an acid-fast bacillus and characterized by the formation of nodules on the surface of the body and especially on the face or by the appearance of tuberculoid macules on the skin that enlarge and spread and are accompanied by loss of sensation followed sooner or later in both types by involvement of nerves with eventual paralysis, wasting of muscle, and production of deformities and mutilations), suggesting they understood that disease had some relationship to an unseen cause. Likewise, there is good evidence that the Romans recognized that leguminous plants enriched for soil productivity and practiced crop rotations with leguminous plants. However, no one really understood the involvement of microscopic organisms in these phenomena.

Although Robert Hooke reported on the fruiting structures of molds in 1664, we most often think of the Dutch microscope builder, Anton van Leeuwenhoek-with his newly constructed microscope-as the first individual to describe microorganisms. In his paper to the Royal Society of London in 1684, van Leeuwenhoek reported the presence of "wee animalcules" in pond water. These observations were confirmed by others, but little was known about these organisms and their relation to the environment in which they grew.

The Nineteenth Century

During the nineteenth century, two important questions were answered that would lay the scientific foundations for both microbiology and soil microbiology:

- Does spontaneous generation occur?
- What is the origin of contagious disease?

The eminent French scientist, Louis Pasteur, in a simple and elegant experiment (Fig. 1), demonstrated that it was microorganisms present in the air that were capable of initiating growth in an exposed sterile culture medium. Microbes simply did not arise spontaneously in a suitable medium.

Pasteur's simple experiment effectively settled the controversy surrounding the prominently held theory of spontaneous generation, vigorously debated at the time. Similar experiment were also conducted by Spallanzani, the Italian scientist, and the Englishman Johan Tyndall. Tyndall's original flasks can still be viewed at the Royal Institute near Piccadilly Square in London. The rejection of the theory of spontaneous generation provided the foundation for aseptic (sterile) technique. While conducting his experiments, Tyndall also noted difficulties in trying to sterilize preparations from hay infusions. Further investigations by Tyndall, and also by Ferdinand Cohn of Germany, led to the discovery of the organisms that were producing endospores in these difficult-to-sterilize preparations.

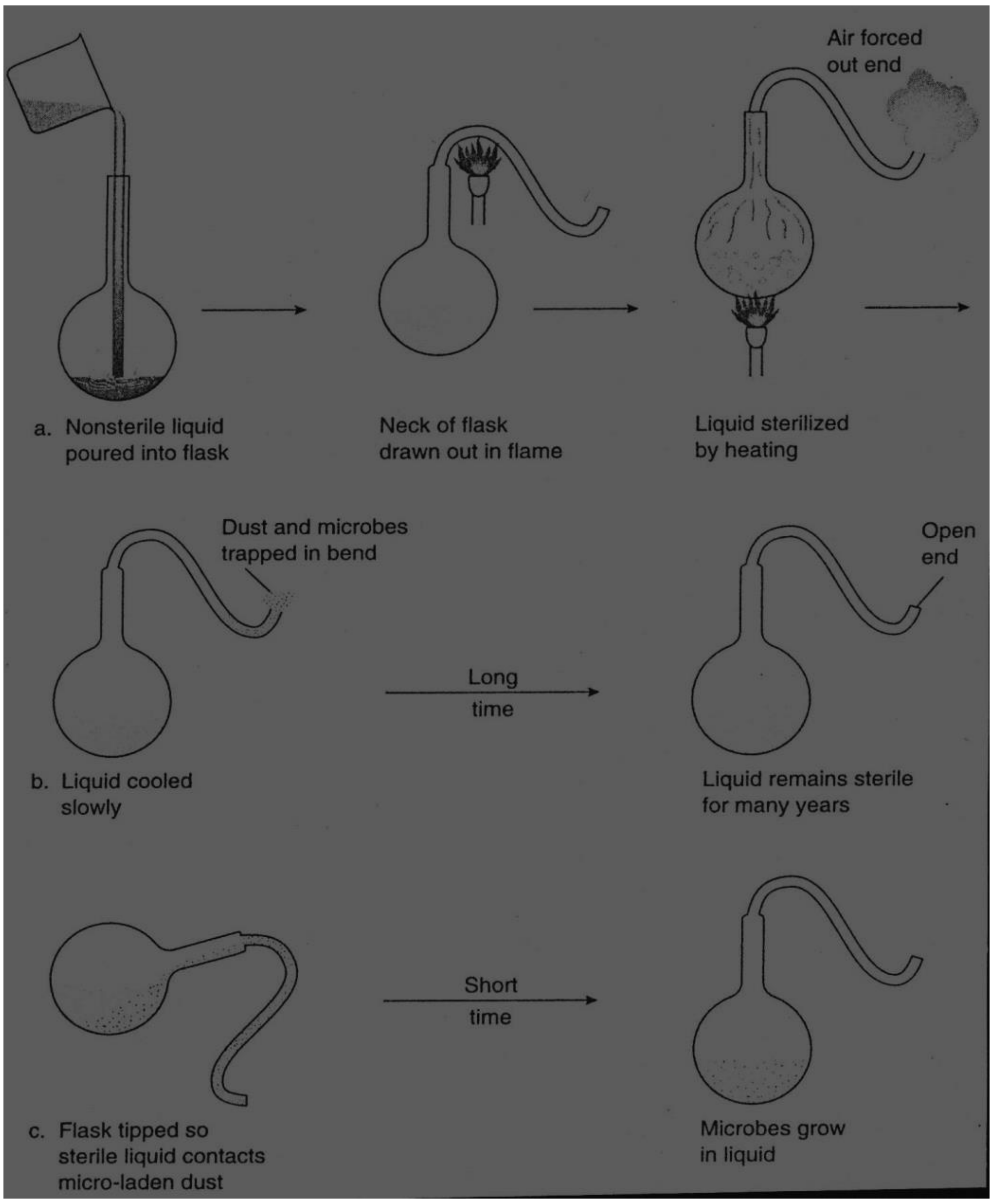


Figure 1: Pasteur's Experiment Disproving Spontaneous Generation.

(a)Pasteur introduced a nonsterile broth into each of two flasks, drew out the flask to a swan-necked shape to provide a dust trap, and sterilized the contents of each by heating. The broth in one flask (b) was not allowed to contact the dust that settled in the trap, and no growth occurred even after a long incubation. After the broth had cooled in the second flask (c) it was brought into contact with dust collecting at the low point near the mouth of the swan neck. After a short period of incubation, the broth became turbid, indicating that growth had occurred in this flask.

During this same period, there was a widely-held belief that disease was caused by something called "contagion". After microorganisms were discovered, they were accepted as the responsible agents, but rigorous proof was lacking. Although Ignaz Semmelweis and Joseph Lister (after whom the product Listerine was named) provided evidence that microorganisms caused human disease, it was not until the seminal work of Robert Koch that the ***germ theory of disease*** had a solid scientific basis. Koch reasoned that to prove that a microorganisms was the causative agent of a disease, the following should apply:

- The organisms should be consistently present in the subject exhibiting the disease symptoms, but not in the healthy subject.
- The organisms should be grown in pure culture away from the subject.
- When the organisms is used to inoculate a healthy susceptible subject, the disease symptoms should appear.
- The organisms should be reisolated from the exposed subject and recultured in the laboratory to confirm its similarity with the original organisms.

The series of steps became known as ***Koch's Postulates,*** which are important for several reasons. First by following these steps, it is possible to demonstrate that a specific organisms is responsible for a specific disease or for some microbiological process (e.g., sulfur oxidation). Second, they suggested the importance of the laboratory culture of organisms, and third, they recognized that specific organisms have specific functions. Acceptance of Koch's Postulates brought the science of microbiology to the point that others could now make specific contributions in more applied fields, such as soil microbiology. Another of Koch's significant developments, reported in 1881, was the use of a gelatin culture medium to study microorganisms *in vitro*. Five years later the Danish physician Christian Gram described a staining procedure based on differential properties of cell walls, leading to important taxonomic decisions. Now soil microorganisms could be studied in a systematic fashion.

In the few remaining years until the turn of the century, significant developments in soil microbiology came in rapid succession. One notable advance was the discovery of ***biological dinitrogen fixation.*** In 1886, Hellrigel and Wilfarth showed that microbial activity in nodules on leguminous plants was able to convert atmospheric nitrogen to a form a plant could use. Subsequently, the Dutch scientist Martinus W. Beijerinck (pronunciation: "buy-a-rink,") isolated the microorganisms responsible for nodulation of leguminous plants and named it *Bacillus radicicola*). In 1989 Frank renamed the bacterium *Rhizobium*, replacing the genus name used by Beijerinck. These actions set in motion a wave of activity in the study of biological nitrogen fixation, which continues to this day.

A second important advance during this period was the discovery of ***chemoautotrophy*** through the study of ***nitrification***. Building on the foundational work of Koch (i.e., that specific organisms have specific effects), Sergei Winogradsky began studying the nitrifying bacteria. He was able to demonstrate that nitrification was really a two-step process, the first being the oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) and the second being the oxidation of NO_2^- to nitrate (NO_3^-). These two steps were mediated by two distinctly different bacteria. Winogradsky isolated representatives of each group and named them, *Nitrosomonas* for the NH_4^+ oxidizers and *Nitrobacter* for the NO_2^- oxidizers. Given the meager understanding of bacterial metabolism at that time, this was a truly remarkable discovery. For these and many other ground-breaking accomplishments, Winogradsky is considered by many to be the Father of Soil Microbiology.

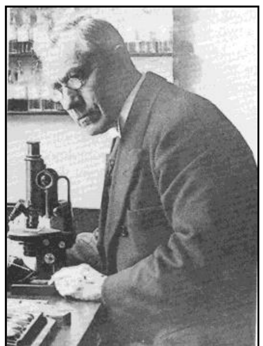
By 1895 developments in microbiology had reached such a point that the Delft School of Microbiology in the Netherlands was established. First headed by Beijerinck-considered by some to be the Father of Microbial Ecology-and then by A.J. Kluyver and C. B. van Niel, the Delft School had a lasting influence on the study of soil microbiology through discoveries in microbial biochemistry, biodiversity, and biotechnology. These individuals posed the following basic questions about microbial physiology that drew the study of microbiology and the environment closer together:

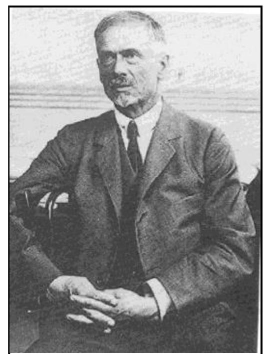
- How do the intact organisms interact with its abiotic and biotic environment?
- How can the fundamental principles of microbiology be brought to bear on applied problems?
- What is the place of microorganisms in the natural world?

The Twentieth Century

If Winogradsky is considered the Father of Soil Microbiology, then Jacob G. Lipman is the founder of American soil microbiology. He considered soil as a complex and living entity which needed to be understood and studied from the standpoint of soil fertility and crop production. This revolutionary concept stands as a milestone in soil microbiology and has been carried across the United States and throughout the world.

Two prominent individuals who shared Lipman's revolutionary concept of soil were Selman A. Waksman and Robert L. Starkey. Besides promoting the "Lipman Philosophy," Waksman's book, *Principles of Soil Microbiology,* and a later work by Waksman and Starkey, *The Soil and the Microbe,* were standard soil microbiology texts for much of the period between 1925 and 1950. However, in many circles Waksman is remembered less for his contributions to soil microbiology than for his discovery of the antibiotic streptomycin, for which he was awarded the 1952 Nobel prize in physiology and medicine. It should be noted that the native environment of the streptomycin-producing organisms (an antinomycete) was the soil! Thus, the quote attributed to Waksman, "From the earth will come our salvation," was prophetic.


Martinus Beijerinck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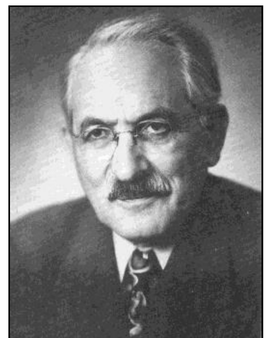
Selman Waksman

Perhaps the greatest contribution of these noteworthy individuals was not so much their scientific papers, published books, inventions, or even patents, but the students they trained, who themselves went on to productive and noteworthy careers. The contributions of these individuals, now too numerous to mention, have enhanced crop production and fostered sound use of the environment worldwide.

Table 3: **Some of the outstanding individuals contributing to the development of soil microbiology.**

Name	Country	Area of contribution
Leeuwenhoek	Netherlands	Inventor of the microscope
Pasteur	France	Repudiation of spontaneous generation, biological nature of nitrification
Tyndall	England	Repudiation of spontaneous generation, understanding of sterilization processes
Cohn	Germany	Understanding of sterilization processes and taxonomy of *Bacillus* (particularly the endospore)
Koch	Germany	Koch's Postulates, gelatin plates for studying soil microorganisms
Winogradsky	Russia	Isolation and taxonomy of chemoautotrophic

		bacteria, especially nitrifiers and sulfur oxidizers
Beijerinck	Netherlands	Isolation of legume root nodulating bacteria, Director of The Delft School of Microbiology
Gram	Denmark	Differential staining procedures
Lipman	USA	Concept of soil as a complex, living entity
Russell	England	Development of importance of soil-plant microorganisms interactions in agriculture and the environment
Starkey	USA	Microbiology of the sulfur bacteria
Waksman	USA (Russia)	Discovery of antibiotics from actinomycetes

Current Topics in soil microbiology:

Following are some of the important research interests that the soil microbiologists are pursuing :

- Symbiotic nitrogen fixation
- Organic matter decomposition (waste removal and composting)
- Mineral nitrogen transformations (nitrification, denitrification and ammonification)
- Rhizosphere studies (root/soil/microorganism interactions)
- Soil enzymes (ureases, cellulases, ligninases, phosphatases)
- Biodegradation and bioremediation
- Metal transformations
- Carbon cycling
- Greenhouse gases and atmospheric pollution (production of methane, carbon dioxide, nitric oxide, nitrous oxide)
- Release and monitoring of genetically engineered microorganisms (GEM)
- Microbial ecology
- Subsurface microbial activity.