

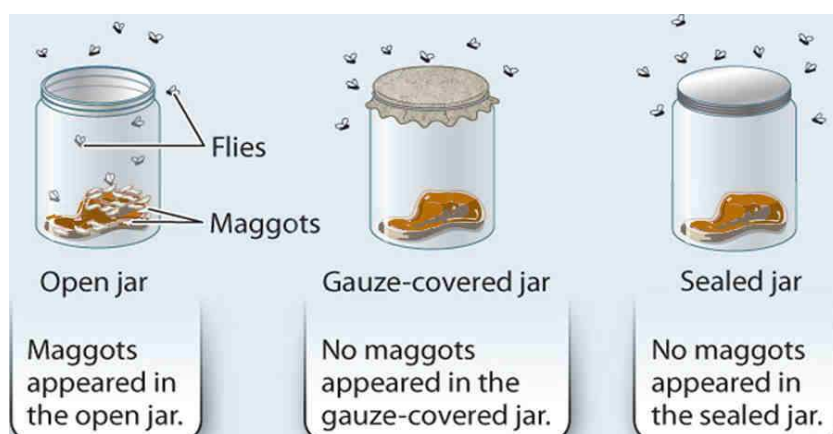
Historical events and contributions of important Microbiologists

The physician Girolamo Fracastoro (1478–1553) suggested that disease was caused by invisible living creatures. In 1665, the first drawing of a microorganism was published in **Robert Hooke's Micrographia**. However, the first person to publish extensive, accurate observations of microorganisms was the **Antony van Leeuwenhoek** (1632–1723) of Netherlands. Leeuwenhoek spent much of his spare time constructing simple microscopes composed of double convex glass lenses held between two silver plates. His microscopes could magnify around 50 to 300 times, and he may have illuminated his liquid specimens by placing them between two pieces of glass and shining light on them at a 45° angle to the specimen plane. This would have provided a form of dark-field illumination in which the organisms appeared as bright objects against a dark background and made bacteria clearly visible

Beginning in 1673, Leeuwenhoek sent detailed letters describing his discoveries to the Royal Society of London. It is clear from his descriptions that he saw both bacteria and protozoa. Little progress was made primarily because microscopic observations of microorganisms do not provide sufficient information to understand their biology. For the discipline to develop, techniques for isolating and culturing microbes in the laboratory were needed.

SPONTANEOUS GENERATION CONTROVERSY

From earliest times, people had believed in spontaneous generation that living organisms could develop from nonliving matter. Even **Aristotle thought some of the simpler invertebrates could arise by spontaneous generation**. This view finally was challenged by the Italian physician **Francesco Redi** (1626–1697), who carried out a series of experiments on decaying meat and its ability to produce maggots spontaneously. Redi placed meat in three containers. One was uncovered, a second was covered with paper (sealed jar), and the third was covered with a fine gauze that would exclude flies. Flies laid their eggs on the uncovered meat and maggots developed. The other two pieces of meat did not produce maggots spontaneously. **Fig.1. Francesco Redi experiment**



However, flies were attracted to the gauze-covered container and laid their eggs on the gauze; these eggs produced maggots as given in figure 1. Thus the generation of maggots by decaying meat resulted from the presence of fly eggs, and meat did not spontaneously generate maggots as previously believed.

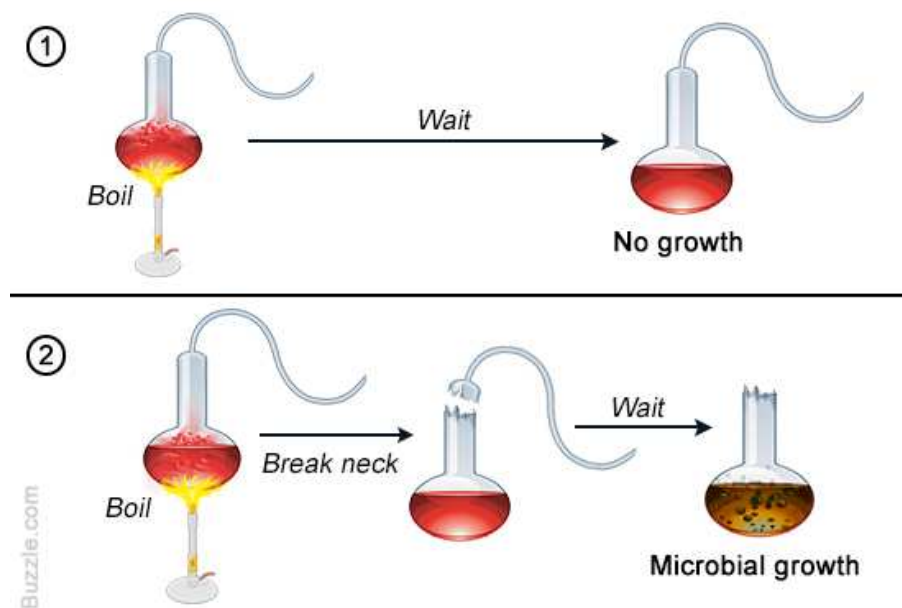
Leeuwenhoek's discovery of microorganisms renewed the controversy. Some proposed that microorganisms arose by spontaneous generation even though larger organisms did not. They pointed out that boiled extracts of hay or meat would give rise to microorganisms after sitting for a while. In 1748, **John Needham** reported the results of his experiments on spontaneous generation. Needham boiled mutton broth and then tightly packed the flasks. Eventually many of the flasks became cloudy and contained microorganisms. He thought organic matter contained a vital force that could confer the properties of life on nonliving matter.

Lazzaro Spallanzani (1729–1799) improved on Needham's experimental design by first sealing glass flasks that contained water and seeds. If the sealed flasks were placed in boiling water for 3/4 of an hour, no growth took place as long as the flasks remained sealed. **He proposed that air carried germs to the culture medium, but also commented that the external air might be required for growth of animals already in the medium.** The supporters of spontaneous generation maintained that heating the air in sealed flasks destroyed its ability to support life.

Louis Pasteur Contribution (Theory of fermentation):

Pasteur demonstrated that fermentation is caused by the growth of micro-organisms, and the emergent growth of bacteria in nutrient broths is due to biogenesis rather than spontaneous generation. Pasteur first filtered air through cotton and found that objects resembling plant spores had been trapped. If a piece of the cotton was placed in sterile medium after air had been filtered through it, microbial growth occurred. Next he placed nutrient solutions in flasks, heated their necks in a flame, and drew them out into a variety of curves, while keeping the ends of the necks open to the atmosphere. Pasteur then boiled the solutions for a few minutes and allowed them to cool. No growth took place even though the contents of the flasks were exposed to the air. Pasteur pointed out that no growth occurred because dust and germs had been trapped on the walls of the curved necks. If the necks were broken, growth commenced immediately. Pasteur had not only resolved the controversy by 1861 but also had shown how to keep solutions sterile. **This was one of the last and most prominent experiments disproving the theory of spontaneous generation.** It also supported germ theory. Although he was not the first to propose germ theory, he developed it and conducted experiments that clearly showed

its correctness and managed to convince most of Europe that it was true. Today, he is often called the father of germ theory.



In, 1856 Monsieur Bigo came to consult Pasteur concerning the difficulty he was having with the alcoholic fermentation of beet sugar in his distillery. Something was going wrong with the process and the alcohol was turning sour. Pasteur decided to go to Mr. Bigo's distillery and have a look at his vats (large tanks). He found that without any reason, the alcoholic fermentation process began to produce lactic acid.

Pasteur decided that there were in fact two kinds of fermentation, each independent of the other, going on in M. Bigo's vats: alcoholic fermentation due to yeast and lactic acid fermentation due to the lactic acid *Bacillus*. When the alcoholic fermentation turned sour it was due to the production of lactic acid by a *Bacillus*. Pasteur discovered and isolated the *Bacillus* and believed that the air was the source of the contamination. Pasteur's experiments proved conclusively that fermentation is caused by microorganisms.

Joseph Lister, was the first to demonstrate the medical significance of Pasteur's work on fermentation and spontaneous generation. Pasteur demonstrated by his experiments that living germs are widely distributed in the air and are the agency of fermentation and putrefaction. When Lister read Pasteur's papers in the early 1860's, he concluded that the inflammation, "pus" and "putrid intoxication" which commonly followed open wounds, was caused by microbes from the air and surrounding surfaces.

Koch Contribution: Germ theory of disease

The germ theory of disease states that some diseases are caused by microorganisms. These small organisms, too small to see without magnification, invade humans, animals, and other living hosts. Their growth and reproduction within their hosts can cause a disease. "Germ" may refer to not just a bacterium but to any type of microorganisms, especially one which causes disease, such as protist, fungus, virus, prion, or viroid. Microorganisms that cause disease are called pathogens, and the diseases they cause are called infectious diseases.

Koch's Postulates

The first direct demonstration of the role of bacteria in causing disease came from the study of anthrax by the German physician Robert Koch (1843–1910). Koch used the criteria proposed by his former teacher, Jacob Henle, to establish the relationship between *Bacillus anthracis* and anthrax. Koch injected healthy mice with material from diseased animals, and the mice became ill. After transferring anthrax by inoculation through a series of 20 mice, he incubated a piece of spleen containing the anthrax bacillus in beef serum. The bacilli grew, reproduced, and produced endospores. When the isolated bacilli or their spores were injected into mice, anthrax developed.

His criteria for proving the causal relationship between a microorganism and a specific disease are known as Koch's postulates. Koch's proof that *B. anthracis* caused anthrax was independently confirmed by Pasteur and his coworkers. They discovered that after burial of dead animals, anthrax spores survived and were brought to the surface by earthworms. Healthy animals then ingested the spores and became ill. Although Koch used the general approach described in the postulates during his anthrax studies, he did not outline them fully until his work on the cause of tuberculosis. In 1884, he reported that this disease was caused by a rod-shaped bacterium, *Mycobacterium tuberculosis*; he was awarded the Nobel Prize in Physiology or Medicine in 1905 for his work. Koch's postulates quickly became the cornerstone of connecting many diseases to their causative agent.

Koch's postulates:

1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
2. The microorganism must be isolated from a diseased organism and grown in pure culture.
3. The cultured microorganism should cause disease when introduced into a healthy organism.
4. The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

Table: Disease caused by microbes and their discoverer

Disease	Organism	Discoverer
Leprosy	<i>Mycobacterium leprae</i>	Hansen
Gonorrhea	Gonococcus	Neisser
Typhoid Fever	<i>Salmonella typhi</i>	Eberth
Tuberculosis	<i>Mycobacterium tuberculosis</i>	Koch
Cholera	<i>Vibrio cholera</i>	Koch
Diphtheria	<i>Corynebacterium diphtheriae</i>	Klebs
Tetanus	<i>Clostridium tetani</i>	Nicolaier
Pneumonia	Pneumococcus	Fraenkel
Dysentery	<i>Shigella shigae</i>	Shiga
Anthrax	Bacillus anthracis	Koch