

Introduction:

Microorganisms are living entities of microscopic size and include bacteria, viruses, yeasts and molds (together designated as fungi), algae, and protozoa. For a long time, bacteria have been classified as prokaryotes (cells without definite nuclei), and the fungi, algae, and protozoa as eukaryotes (cells with nuclei); viruses do not have regular cell structures and are classified separately. Microorganisms are present every where on Earth, including humans, animals, plants and other living creatures, soil, water) . Among the microorganisms, some molds, yeasts, bacteria, and viruses have both desirable and undesirable roles in our food. It is important to isolate them in pure culture and study their morphological, physiological, biochemical, and genetic characteristics. The discovery of microorganisms ran parallel with the invention and improvement of the microscope the first person to see different types of microorganisms, especially bacteria under a microscope was Antony van Leeuwenhoek, he called them animalcules. By 1838, Ehrenberg (who introduced the term *bacteria*) had proposed at least 16 species in 4 genera and by 1875 Ferdinand Cohn had developed the preliminary classification system of bacteria.

WHERE ARE THEY COMING FROM?

“Spontaneous Generation” was an early belief that living things can arise from vital forces present in nonliving and decaying matter.(e.g: maggots from meat or mushrooms from rotting wood).

However, 1665, Redi disproved that theory by showing that the maggots in spoiled meat and fish could only appear if flies were allowed to contaminate them. The advocates of the spontaneous generation theory argued that the animalcules could not regenerate by themselves (biogenesis), but they were present in different things only through abiogenesis (spontaneous generation). In 1749, Turbevill Needham showed that boiled meat and meat broth, following storage in covered flasks, could have the presence of animalcules within a short time. This was used to prove the appearance of these animalcules by spontaneous generation. Lazzaro Spallanzani (1765) showed that boiling the meat infusion in broth in a flask and sealing the flask immediately prevented the appearance of these microscopic organisms, thereby disproving Needham's theory. Theodore Schwann [(1838), by passing air through red-hot tubes], and

Schroeder [(1854), by passing air through cotton] showed that bacteria failed to appear in boiled meat infusion even in the presence of air.

Finally, in 1861, Louis Pasteur demonstrated that, in boiled infusion, bacteria could grow only if the infusions were contaminated with bacteria carried by dust particles in air. His careful and controlled studies proved that bacteria were able to reproduce (biogenesis) and life could not originate by spontaneous generation.

- trapped airborne organisms in cotton;
- he also heated the necks of flasks, drawing them out into long curves, sterilized the media, and left the flasks open to the air;
- no growth was observed because dust particles carrying organisms did not reach the medium, instead they were trapped in the neck of the flask; if the necks were broken, dust would settle and the organisms would grow; in this way Pasteur disproved the theory of spontaneous generation

The alternative hypothesis that living organisms can arise only from preexisting life forms is called “Biogenesis.

Food microbiology:

Is the study of the microorganisms that inhabit, create, or contaminate food (Including the study of microorganisms causing food spoilage), and preservation of food.

"Good" bacteria, however, such as probiotics, are becoming increasingly important in food science; in addition, microorganisms are essential for the production of foods such as cheese, yogurt, other fermented foods, bread, beer and wine.

What should a food microbiologist know?

- Characteristics of the different types of microbes
- How to identify and enumerate them
- Factors that affect their growth (innate and introduced)
- Fermentation vs spoilage
- How microbes cause disease
- That the field of food microbiology is a work in progress!

Food safety:

Unsafe food has been a human health problem since history was first recorded, and many food safety problems encountered today are not new. Although governments all over the world are doing their best to improve the safety of the food supply, the occurrence of food-borne disease remains a significant health issue in both developed and developing countries. It has been estimated that each year 1.8 million people die as a result of diarrheal diseases and most of these cases can be attributed to contaminated food or water. Proper food preparation can prevent most food-borne diseases.

Key recommendations for food safety:

- (1) Keep clean (Clean hands, food-contact surfaces, fruits and vegetables).
- (2) Keep fruits and vegetables separate from raw meat, poultry and seafood while shopping, preparing or storing them.
- (3) Cook thoroughly (Cook foods to a safe temperature to kill microorganisms).
- (4) Refrigerate perishable foods promptly and defrost foods properly.
- (5) Use safe water and raw materials (Avoid raw (unpasteurized) milk or milk products, raw or partially cooked eggs and foods containing raw eggs, raw and undercooked meat and poultry).

Probiotic (Good bacteria):

“live microorganisms , which, when administered in adequate amounts, confer a health benefit on the host.”

The first use of the term “probiotic” as microorganisms that have effects on other microorganism was accredited to Lilly and Stilwell (1965), expressed as follows: Substances secreted by one microorganism that stimulate another microorganism. Later, the definition was greatly improved by Fuller in 1989, whose explanation was very close to the definition used today. Fuller in 1989 described probiotics as "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". Lactic acid bacteria (LAB) and Bifidobacteria are the most common types of microbes used as probiotics, but certain yeasts and bacilli may also be used. Probiotics are commonly consumed as part of fermented foods with specially

added active live cultures, such as in yogurt, soy yogurt, or as dietary supplements. Probiotics are also delivered in fecal transplants, in which stool from a healthy donor is delivered like a suppository to an infected patient. Potential Uses for Infectious diarrhea, Antibiotic Associated Diarrhea (*Clostridium difficile*) , Inflammatory bowel disease (Traveler's diarrhea, Prevention of Necrotizing enterocolitis) allergy & Irritable Bowel Syndrome).

You'll find probiotics in fermented foods such as yogurts, sauerkraut, and kimchi. Keep in mind that most of the time, you can't get enough probiotics through eating foods alone, and you'll need to take a supplement.

Factors affecting microbial growth in food:

There are basically two parameters that effect the growth of microorganisms in food products, extrinsic & intrinsic.

Extrinsic parameters are those properties of the environment (processing & storage) that exist outside of the food product which affect both the foods & their microorganisms.

While ***intrinsic parameters*** are properties that exist as part of food product itself. By understanding the factors affecting the growth of microorganisms in food we can know how to keep food safe to eat. This knowledge can also help us to work out how to preserve food for longer. The following common intrinsic factors affect the growth and multiplication of M.O in foods:

- pH.
- Presence and availability of water.
- Oxidation-reduction potential.
- Nutrient content.
- Presence of antimicrobial substances.

pH:

pH indicates the hydrogen ion concentrations in a system and is expressed as: -log [H⁺], the (negative logarithm of the hydrogen ion or proton concentration). It ranges from 0 to 14, with 7.0 being neutral pH. [H⁺] concentrations can differ in a system, depending on what acid is present. Growth of microorganisms is affected by the pH of growth environments in food (growth medium) which is the result of large number of enzymes responsible for metabolism and growth. Most bacteria grow best at about pH 7 and grow poorly or not at all below pH 4.

Yeasts and molds, therefore, predominate in low pH foods where bacteria cannot compete. The lactic acid bacteria are exceptions; they can grow in high acid foods and actually produce acid to give us sour milk, pickles, fermented meats, and similar products.

Water availability:

Is a measure of the availability of water for biological functions and relates to water present in a food in free form. Available water for microorganisms varies depending on microorganism and the type of food. In general, bacteria require more water activity than molds and yeasts. Most bacteria grow well in a medium with a water activity approaching 1.0. Gram negative bacteria have higher water requirements than gram positive bacteria. Most of the food spoilage bacteria do not grow below a a_w 0.91, while spoilage molds can grow even at a a_w 0.80. The aerobic food poisoning bacterium, *Staphylococcus aureus* is found to grow at a a_w as low as 0.86 while anaerobic *Clostridium botulinum* does not grow below a a_w 0.94.

Redox potential (O/R, Eh):

Measures the potential difference in a system generated by a coupled reaction in which one substance is oxidized and a second substance is reduced. Microorganisms display varying degrees of sensitivity to the oxidation-reduction potential (O/R, Eh) of their growth medium. Aerobic microorganisms require positive Eh values (oxidized) for growth, whereas anaerobes require negative Eh values (reduced). The Eh of a medium can be reduced by microorganisms by their production of certain metabolic byproducts such as H₂S, which has the capacity to lower Eh to -300 mV. Because H₂S reacts readily with O₂; it will accumulate only in anaerobic environments. With regard to the Eh of foods, plant foods, especially plant juices, tend to have Eh values of from 300 to 400. It is not surprising to find that aerobic bacteria and molds are the common cause of spoilage of products of this type. Solid meats have Eh values of around -200 mV; in minced meats, the Eh is generally around 200 mV. Cheeses of various types have been reported to have Eh values on the negative side, from -20 to around -200 mV.

Nutrient content:

In order to grow and function normally, the microorganisms of importance in foods require the following: water, source of energy, source of nitrogen, vitamins and related growth factors & minerals.

Microorganisms normally present in food vary greatly in nutrient requirements, with bacteria requiring the most, followed by yeasts and molds. Microorganisms also differ greatly in their ability to utilize large and complex carbohydrates (e.g., starch and cellulose), large proteins (e.g., casein in milk) , and lipids. Microorganisms capable of using these molecules do so by producing specific extracellular enzymes (or exoenzymes) and hydrolyzing the complex molecules to simpler forms outside before transporting them inside the cell. Microbial cells, following death and lysis, release intracellular enzymes that can also catalyze breakdown of complex food nutrients to simpler forms, which can then be utilized by other microorganisms.

Antimicrobial Constituents:

The stability of some foods against attack by microorganisms is due to the presence of certain naturally occurring substances that have been shown to have antimicrobial activity.

Cow's milk contains several antimicrobial substances, including lactoferrin, conglutinin, and the lactoperoxidase system.

Eggs contain lysozyme, as does milk, and this enzyme, along with conalbumin, provides fresh eggs with a fairly efficient antimicrobial system.

Ovotransferrin appears to be the inhibitory substance in raw egg white that inhibits *Salmonella enteritidis*.