Department of Food Science

Food Safety

FSE 99-21

Basic Food Microbiology

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Introduction

Microorganisms are tiny, mostly one-celled organisms capable of rapid reproduction under proper growth conditions. Those microorganisms important in the food industry include the *bacteria, viruses, yeasts, molds,* and *protozoans.*

Many are helpful and serve useful functions such as causing breads to rise, fermenting sugars to alcohol, assisting in the production of cheese from milk, and decaying organic matter to replenish nutrients in the soil. Microorganisms can also cause foods to spoil and make them inedible. Spoilage organisms cost the food industry millions of dollars each year. Microorganisms can also be harmful. These are called *pathogens* and cause between 24 to 81 million cases of foodborne illness in the U.S. each year.

These forms of life, some so small that 25,000 of them placed end to end would not span one inch, were little known until the last century. Antony van Leeuwenhoek and others discovered "very little animalcules' in rain water viewed through crude microscopes. We now know that microorganisms occur everywhere on the skin, in the air, in the soil, and on nearly all objects.

It was not until Pasteur proved that microorganisms could be eliminated from a system, such as a can of food, and sealed out (*hermetically sealed*), that man could exert control over the microbes in his environment.

Terminology

Bacteria are single-celled microorganisms found in nearly all natural environments. Outward appearances of the cell such as size, shape, and arrangement are referred to as *morphology*. Morphological types are grouped into the general categories of spherical (the cocci), cylindrical (the rods) and spiral. The cocci may be further grouped by their tendencies to cluster. Diplococci attach in pairs, streptococci in chains, staphylococci bunch like grapes, and sarcinae produce a cuboidal arrangement. Bacterial cells have definite characteristic structures such as the *cell wall*, *cytoplasm*, and nuclear structures. Some also possess hairlike appendages for mobility called *flagella*, *fimbriae* which aid in attachment, plus *cytoplasmic* and *membranous inclusions* for regulating life processes.

Viruses are extremely small parasites. They require living cells of plants, animals, or bacteria for growth. The virus is mainly a packet of genetic material which must be reproduced by the host.

Yeast and mold are *fungi* which do not contain chlorophyls. They range in size from single-celled organisms to large mushrooms. Although some are multi celled, they are not differentiated into roots, stems and leaves. The true fungi produce masses of filamentous *hyphae* which form the *mycelium.* Depending on the organism, they may reproduce by fission, by budding as in the case of yeasts, or by means of *spores* borne on fruiting structures depending on the organism.

Protozoa are single-celled organisms such as the amoeba which can cause disease in humans and animals. They possess cell structure similar to higher, more complex organisms.

Microorganisms are referred to by their scientific names which are often very descriptive. The first part of the name, the genus, is capitalized such as *Streptococcus*, spherical cells which occur in strips, *Lactobacillus* which are rod-shaped organisms commonly found in milk, or *Pediococcus* spherical cells which ferment pickles. The second part of the name is not capitalized and gives added information. Both parts of the name are underlined or italicized as in the case of *Saccharomyces cerevisiae*, a yeast which commonly ferments sausage.

The Cell

The cell is the basic unit of life. Our bodies are made up of millions of cells, but many microorganisms are single celled creatures. Cells are basically packages of living matter surrounded by membranes or walls. Within the cell are various organelles which control life processes for the cell such as intake of nutrients, production of energy, discharge of waste materials, and reproduction.

Growth of the cell normally means reproduction. Bacteria and similar organisms reproduce by *binary fission*, a splitting of a single cell into two. The control center for the bacterial cell is the *nuclear structure.* Within it is the genetic material which is duplicated and transferred to daughter cells during reproduction. These daughter cells can again divide to produce four cells from the original one. The time It takes for a new cell to produce a new generation of daughter cells is called *generation time.* Under optimum growth conditions, certain organisms can have a generation time of 15 minutes. In four hours over 65,000 cells could be produced from a single microorganism!

Under adverse conditions, certain bacteria can protect the cell's genetic material by producing *spores*. These are extremely resistant capsules of genetic materials. Though there are no discernible life processes in the spore, under proper sporulation conditions, a viable, reproducing cell will germinate from it.

Factors Affecting Growth

Microorganisms, like other living organisms, are dependent on their environment to provide for their basic needs. Adverse conditions can alter their growth rate or kill them. Growth of microorganisms can be manipulated by controlling:

- Nutrients available
- Oxygen
- Water
- Temperature
- Acidity and pH
- Light
- Chemicals

Nutrients

Nutrients such as carbohydrates, fats, proteins, vitamins, minerals and water, required by, man are also needed by microorganisms to grow. Microbes differ in their abilities to use *substrates* as nutrient sources. Their enzyme systems are made available according to their genetic code. They vary in ability to use nitrogen sources to produce amino acids and, therefore, proteins. Some require amino acids to be supplied by the substrate. When organisms need special materials provided by their environment, we refer to them as *fastidious.* Difference in the utilization of nutrients and the waste products they produce are important in differentiating between organisms.

Oxygen

Microbes also differ in their needs for free oxygen. *Aerobic* organisms must grow in the presence of free oxygen and *anaerobic* organisms must grow in the absence of free oxygen. *Facultative* organisms can grow with or without oxygen, while *microaerophilic* organisms grow in the presence of small quantities of oxygen.

Water

Water is necessary for microbes to grow, but microbes cannot grow in pure water. Some water is not available. A measurement of the availability of water is a_w or *water activity.* The a_w of pure water is 1.0 while that of a saturated salt solution is 0.75. Most spoilage bacteria require a minimum a_w of 0.90. Some bacteria can tolerate an a_w above 0.75 as can some yeasts and most molds. Most yeasts require 0.87 water activity. An a_w of 0.85 or less suppresses the growth of organisms of public health significance.

Temperature

Microorganisms can grow in a wide range of temperatures. Since they depend on water as a solvent for nutrients, frozen water or boiling water inhibits their growth. General terms are applied to organisms based on their growth at different temperatures. Most organisms grow best at or near room and body temperature. These are *mesophiles.* Those growing above 40^0C (105^0F) are called *thermophiles* while those growing below 25^0C(75^0F) are called *psychrotrophs.*

Acidity

The nature of a solution based on its acidity or alkalinity is described as pH. The pH scale ranges from 0, strongly *acidic*, to 14, strongly *basic. Neutral* solutions are pH 7, the pH of pure water. Most bacteria require near neutral conditions for optimal growth with minimums and maximums between 4 and 9. Many organisms change the pH of their substrate by producing by-products during growth. They can change conditions such that the environment can no longer support their growth. Yeasts and molds are more tolerant of lower pH than the bacteria and may outgrow them under those conditions.

Light & Chemicals

Ultraviolet light and the presence of chemical inhibitors may also affect the growth of organisms. Many treatments such as hydrogen peroxide and chlorine can kill or injure microbes. Under certain conditions those given a sublethal treatment are injured, but can recover.

Growth

Characteristic growth patterns can be illustrated on a graph. There is a selected portion of the normal growth curve which is referred to as the *logarithmic growth phase* or the *log phase.* When cells begin to grow, we usually observe a period of no apparent growth which we refer to as the *lag phase.* This occurs because cells are making necessary adjustments to adapt. Next we experience the rapid growth or the *log phase* previously described. As cell mass becomes large, nutrients are exhausted and metabolic byproducts collect. Growth tapers off and the population remains constant for a time. This is referred to as the *stationary phase* of growth. With no intervention in the system the population will enter a *death phase* and total numbers of organisms will decline.

Enumeration of Cells

Numbers of microorganisms can be estimated based on cell counts, cell mass, or activity. A *direct count* of cells may be made by examination of a known volume of cell suspension under a microscope. This method is rapid and requires minimal equipment. This does not distinguish living cells from dead and may be tedious. The application of certain stains make visible morphological characteristics of the organism which can aid in identification.

Probably the most common method of enumerating cells is the *plate count.* A known volume of a diluted specimen is added to agar in a petri dish. Assuming dilute solution and that each organism will divide until it develops a visible mass or *colony*, the colonies can be counted and multiplied by the dilution factor to estimate the number of organisms in the original sample. This method is based on the assumption that a colony forming unit (CFU) is a single organism. This will not hold exactly true in the case of strips or clumps of cells. Sublethally injured organisms may not grow. The culture conditions may not be conducive to the growth of certain types of *fastidious organisms* such as anaerobes.

To measure the progress of a culture in a clear broth, changes in *turbidity* can be measured and related to numbers or organism. This method is easy and rapid. Many commercial establishments have found application for this method.

Identification

As many types of cells look similar in morphology and produce similar colonies, it becomes necessary to identify the organisms by their biochemical characteristics. Biochemical testing requires pure cultures isolated from a single colony from a plate count or streaked plate made for isolation purposes. *Isolates* are grown in an enriched broth to produce large cell numbers. Various media can then be inoculated with the culture and then growth can be observed by carefully formulating the various media, the biochemical and growth characteristics of the organism can be determined. Previously determined morphological characteristics can be combined with biochemical data to properly classify the organism. Newer methods more rapidly identify organisms of interest using other characterization such as monoclonal antibodies and DNA.

Thermal Processing of Foods

Low-acid canned foods are regulated by 21CFR113. These foods have a pH of greater than 4.6 and have a a_w greater than 0.85. the regulations require that a *scheduled process* established by a processing authority be selected by the manufacturer which renders the product, under the specified conditions, *commercially sterile.* Commercial sterility is determined by processing food inoculated with known quantities of microbial spores. The test organisms used should simulate the resistance of *Clostridium botulinum* under those conditions. A process which eliminates these spores will destroy *Clostridium botulinum* spores.

Acid and acidified foods will not allow the growth of *Clostridium botulinum.* However, a *pasteurizing* heat treatment is necessary to destroy other bacteria, viruses, yeasts, and mold. Temperatures and process times which destroy microorganisms without destruction of nutrients may fail to deactivate enzymes. If the process selected does not inactivate the enzymes, product changes may proceed during storage, at an accelerated rate, and cause a loss of product quality. The microbiological quality of raw product is the simple greatest determinant in the level of quality in the food. Thermal processing is no substitute for good raw product quality.