MODULE 2 - BACTERIAL GROWTH

Objective

On completion of this module, participants will be able to:

- o Identify factors essential for the growth of microorganisms
- Relate the requirements for bacterial growth to the definition of "Potentially hazardous food"
- Discuss the types of illness associated with food poisoning

Most food preservation techniques used by processors employ knowledge of factors that affect the growth of bacteria. Nutrients, temperature, water activity, pH, chemical inhibitors, and atmosphere all can be used to control growth. Each of these will be addressed individually.

Factors Affecting Growth:

Nutrients

Inhibitors

Time/Temperature

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Water Activity

Atmosphere

The ServSafe ® food worker training program of the Educational Foundation of the National Restaurant Association uses the acronym FATTOM to relate the same concepts -

Food

Acidity

Temperature

Time

Oxygen

Moisture

For our discussion, we'll use the terminologies above.

Nutrients

Bacteria, like any living organism require food and water to carry on their life processes. Nutrients must be in solution before they can be transported into the cells, so water is essential. In general, bacteria also require sources of carbon, nitrogen, sulfur and phosphorous. Some microorganisms have the necessary enzyme systems to transform these few simple materials into the complex substances required for their life processes, while other microorganisms require certain preformed compounds.

The specifics of the nutrient requirements and the actual mechanisms involved in nutrient transport are important and interesting areas of study. But, unless you are a microbiologist or biochemist the details of this can be rather complicated and tedious. From a practical standpoint, since microorganisms require nutrients to grow and proliferate, *proper sanitation* is essential to eliminate food residues, especially on food contact surfaces. Additionally, since microorganisms require nutrients to be in solution for transport into the cell, it is important that the food-processing environment is constructed to prevent the accumulation of standing water.

Bacteria have phenomenal growth rates. They grow through a process of binary fission - splitting in two every 20 to 30 minutes under optimal conditions. We can follow bacterial growth through a 4-phase growth cycle.

Lag Phase:

This is the first phase, where cells may increase in size but the actual number of cells does not increase. Here the bacteria adjust their metabolism to the environment. This occurs when there is a drastic change in temperature or when the bacteria are moved from one medium to another. The lag phase is the phenomenon of bacterial growth that allows the use of "time as a public health control" in the Food Rules.

Logarithmic Growth Phase:

Also known as the **Log Phase**. Cells actively divide by simple fission: one cell becomes two, two become four, and so on. During this phase the bacteria experience rapid exponential growth provided the necessary

conditions of moisture, warmth and nutrients are present. The time required for a cell to grow and then divide into two cells is termed the generation time or doubling time.

Stationary Phase:

Cell numbers remain constant. Cell growth and cell death are in balance because they are beginning to deplete nutrients and accumulate waste products.

Death Phase:

Cell numbers begin to decline as a result of ongoing depletion of nutrients and the accumulation of toxic metabolic by-products.

The lag phase is very important. If food is handled properly, the bacteria are kept in this phase and not allowed to multiply. Proper sanitation is important to limit available nutrients and thereby prevent bacterial growth.

Temperature

Another essential factor that affects the growth of bacteria is temperature. Microbial growth can occur over a wide range of temperatures from about 14°F to 194°F. Organisms are divided into three groups based on their temperature growth range:

	Temperature		
	Optimum	Range	
Psychrophile	< 68°F	32° - 86°F	
Mesophile Includes psychrotrophs	98°F	50° - 110°F	
Thermophile	131°F	110° - 194°F	

Psychrophiles grow at or near refrigeration temperatures or 32°F - 86 °F. Mesophiles grow at or near room temperatures, 50°F - 110°F. Thermophiles grow at hot temperatures above 110 °F. In addition to these three terms, there is also the designation <u>*psychrotroph*</u>. Their optimal growth temperature is in the mesophilic range but they are capable of growth at refrigeration temperatures.

Most of the microorganisms of public health concern in foods are mesophiles and their optimum growth temperature corresponds to human body temperature. Typically, the higher the temperature (within the normal growth range), the more rapid the growth of the organism. This can be explained by the fact that growth is catalyzed by enzymatic reactions and the rule of thumb is that with every 18°F rise in temperature, the catalytic rate of an enzyme doubles.

Time/Temperature

It is not just the temperature that is the problem; it is the total time of exposure at these temperatures that needs to be controlled. The goal is to minimize the *time* of exposure of foods to temperatures in the mesophilic range. It is recommended that foods be kept below 45°F or above 140°F. In many situations it may be impossible to completely avoid product exposure to mesophilic temperatures.

Water Activity (a_w)

Water (a_w) activity refers to the availability of water to the organism. Water activity is directly related to the vapor pressure of the water in a solution and is determined by measuring the equilibrium relative humidity of the air over the solution in a closed container. Relative humidity divided by 100 equals the water activity.

$(a_w) = RH/100$

If you have a closed container of water, the air over the water becomes saturated. The relative humidity is 100% which equals an a_w of 1.0. So water has a water activity of 1.0. Foods are more complex systems so not all the water in the food is available to microorganisms. Think of water activity as water availability. Water molecules are loosely oriented in pure water and they are readily available to microorganisms. When we add substances like salt and sugar, the water molecules orient themselves to the added substance, and the properties of the entire solution change. The water is bound, and less available to microorganisms.

Foods vary in their water activity. See Table 1: Principal Groups of Foods Based on $A_{w_{\!\scriptscriptstyle \! \! \! M}}$

Table 1

0.98 and above	Below 0.98 to 0.93	Below 0.93 to 0.85	Below 0.85 to 0.60	Below 0.60	
Fresh meats and fish Fresh fruits and vegetables Milk and other beverages Canned vegetables in brine Canned fruit in light syrup	0.93 Evaporated milk Tomato paste Lightly salted fish, pork and beef products Canned cured meats Fermented sausages (not dried) Cooked sausages Processed cheese Gouda cheese Canned fruits in heavy syrup	0.85 Dry or fermented sausage Dried beef Raw ham Aged cheddar cheese Sweetened condensed milk	0.60 Intermediate moisture foods Dried fruits Flour Cereals Jam and jellies Molasses Heavy salted fish Meat extract Nuts	Confectionery Chocolate Honey Noodles Crackers Potato Chips Dried egg, milk, and vegetables	
	Bread				
Microbial Ecology of Foods, Volume 1, Factors Affecting Life and Death of Microorganisms.					
International Commission on Microbiological Specifications for Foods. Academic Press, 1980.					

Principal Groups of Foods Based on aw

Most bacteria, including those of public health significance, will not grow when the water activity is 0.85 or less. Many yeast and molds can grow below this level but this is a spoilage concern and not a food safety concern.

Inhibitors

Foods can contain chemicals that are either natural or added that restrict or prevent the growth of microorganisms. Salt is a good example of an added chemical that can inhibit the growth of bacteria. Chemical preservatives like sodium nitrite, sodium benzoate and calcium propionate can also inhibit the growth of microorganisms.

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Another factor that can control the growth of bacteria is pH. pH is expressed as the negative logarithm of the hydrogen ion concentration.

$[pH = (-log of the [H^{+}])]$

That is, the pH shows how acid a food is. Most bacteria don't grow very well in acid foods. pH ranges from 0 to 14, with 7 being neutral. Foods with a pH of 4.6 and below are considered acid foods, like most fruit juices. Foods with a pH above 4.6 are said to be low acid, like meats and vegetables.

pH RANGES FOR GROWTH OF MICROORGANISMS		
Bacteria (gram+)	4.0 to 8.5	
Bacteria (gram -)	4.5 to 9.0	
Molds	1.5 to 9.0	
Yeast	2.0 to 8.5	

Microorganisms can only grow at certain pH levels. Mold and yeast can grow over a broad range of pH. Bacteria are more restricted. Gram positive bacteria grow in a pH range of 4 to 8.5 and Gram negative grows between 4.5 and 9.0. So you can use pH to control the growth of bacteria.

Food is considered to be in a safe pH range - or shelf stable - when the final pH is 4.6 or below.

Atmosphere

Some bacteria require a specific type of atmosphere for growth. Microorganisms are categorized as aerobes, anaerobes, facultative anaerobes and microaerophilic.

Aerobes require oxygen and include such bacterial genera as Bacillus.

Anaerobes grow only in the absence of molecular oxygen. These organisms include clostridia.

Facultative anaerobes, which include most of the other food borne pathogens, can grow whether the environment has oxygen or not.

Microaerophilic is a term applied to organisms, which grow only in reduced oxygen environments.

It is important to understand that microbial pathogens are associated with all of the groups mentioned above. Knowledge of the atmospheres surrounding the food is an especially important consideration in determining which pathogens are likely to be a problem.

Atmospheres & Packaging

Many of the organisms that spoil foods are aerobic. Technologies that are used to extend shelf life do so by altering the atmospheric environment of the food package to prevent the growth of aerobic organisms. These technologies include vacuum packaging, controlled atmosphere packaging, and modified atmospheric packaging. Most pathogens are facultative anaerobes so attempts to control spoilage by changing the atmosphere from aerobic to anaerobic or somewhere in between can be potentially dangerous because by eliminating the competing aerobic flora it can select for pathogens and inhibit those microorganisms that give the tell tale signs of spoilage. A product may not appear spoiled but may be toxic.

POTENTIALLY HAZARDOUS FOOD

DEFINITION

The Food Rules define "potentially hazardous food" as:

any food that consists in whole or in part of milk or milk products, eggs including shell eggs, meat, poultry, fish, molluscan shellfish, edible crustacea or other ingredients, including synthetic ingredients, in a form capable of supporting rapid and progressive growth of infectious or toxigenic microorganisms or the growth and toxin production of Clostridium botulinum. The term includes foods associated with foodborne illness such as cooked rice, cooked pasta, garlic-in-oil preparations, baked potatoes, and cut melons. The term does not include foods which have a pH level of 4.6 or below; or a water activity (a_w) of 0.85 or less; or food products in hermetically sealed containers processed to achieve and maintain commercial sterility under unrefrigerated conditions.

The **2001 FDA Food Code** even more specifically defines "Potentially hazardous food" as:

a FOOD that is natural or synthetic and that requires temperature control because it is in a form capable of supporting:

(i) The rapid and progressive growth of infectious or toxigenic microorganisms;

(ii) The growth and toxin production of *Clostridium botulinum*; or

(iii) In raw shell eggs, the growth of **Salmonella Enteritidis**.

(b) **"Potentially hazardous food"** includes an animal FOOD (a FOOD of animal origin) that is raw or heat-treated; a FOOD of plant origin that is heat-treated or consists of raw seed sprouts; cut melons; and garlic-in-oil mixtures that are not modified in a way that results in mixtures that do not support growth as specified under Subparagraph (a) of this definition.

(c) "Potentially hazardous food" does not include:

(i) An air-cooled hard-boiled egg with shell intact;

(ii) A FOOD with an a_w value of 0.85 or less;

(iii) A FOOD with a pH level of 4.6 or below when measured at 24°C (75°F);

(iv) A FOOD, *in an unopened* HERMETICALLY SEALED CONTAINER, that is commercially processed to achieve and maintain commercial sterility under conditions of nonrefrigerated storage and distribution;

(v) A FOOD for which laboratory evidence demonstrates that the rapid and progressive growth of infectious or toxigenic microorganisms or the growth of **S. Enteritidis** in eggs or **C. botulinum** can not occur, such as a FOOD that has an a_w and a pH that are above the levels specified under Subparagraphs (c)(ii) and (iii) of this definition and that may contain a preservative, other barrier

to the growth of microorganisms, or a combination of barriers that inhibit the growth of microorganisms; or

(vi) A FOOD that does not support the growth of microorganisms as specified under Subparagraph (a) of this definition even though the FOOD may contain an infectious or toxigenic microorganism or chemical or physical contaminant at a level sufficient to cause illness.

DISCUSSION

From the definition, you can see that key points in determining that a food is "potentially hazardous" is its water activity and pH. Any food with a pH AT OR BELOW 4.6, **OR** a water activity AT OR BELOW 0.85 is, by definition, not a potentially hazardous food.

This does not mean such non-potentially hazardous foods are safe under all conditions. Any food, including water and ice, can be contaminated with pathogenic microorganisms. The designation simply means that the food will not support the "rapid and progressive" growth of pathogenic bacteria when held at non-refrigeration temperatures. The term itself gets even blurrier when you consider that some pathogenic bacteria continue to grow (multiply) even at refrigeration temperatures in the low to mid - 30 degree F range. See, for example, Listeria monocytogenes in the next module on Foodborne Bacterial Pathogens and Thermal Destruction.

TYPES OF FOODBORNE ILLNESS

There are two primary types of foodborne illness, an infection and an intoxication; and a third type that combines the two. The bacteria that cause these are different. They're all considered pathogens but the way they make a person sick is different.

• Foodborne Infection

A foodborne infection occurs when the microorganism itself is ingested with the food. The organism establishes itself in the host's body and multiplies. Since the infection is a consequence of growth in the body, the time from ingestion until symptoms occur is relatively long. Foodborne illness caused by Salmonella bacteria is an example of an infection.

• Foodborne Intoxication

A foodborne intoxication occurs when specific pathogenic bacteria grow in the food and releases toxins into the food that is subsequently consumed. It is the toxin that makes the person sick. Since the illness is a consequence of absorption of the preformed toxin by the intestinal tract, and not microbial growth in the host's body, symptoms of intoxication have a much more rapid onset than foodborne infections. Foodborne illness caused by the enterotoxin produced by Staphylococcus aureus is an example of an intoxication.

 \circ Toxicoinfection

A third type is called toxicoinfection, which combines the two. This is also sometimes called a toxin-mediated infection. Toxicoinfections are characterized by bacteria that are non-invasive and cause illness by producing toxins *while growing* in the human intestines. The times of onset are generally, but not always, longer than those for intoxications, but less than those for infections. Foodborne illness caused by E. coli O157:H7 is an example of a toxicoinfection.

Complicating factors

Individual resistance: The infective dose of any given pathogen is not a constant. Individuals vary in their own health status. It is not unusal in a foodborne outbreak for several people who ate the suspect food to have no symptoms.

Carriers: Some people can shed the pathogen without exhibiting any symptoms themselves. Mary Mallon (Typhoid Mary) was a real person who worked as a cook in New York state until she was "hospitalized for life" to prevent her continuing to work as a cook.

Dual activity bacteria: Bacillus cereus can cause an intoxication or can cause an infection.

EPIDEMIOLOGY SUMMARY TABLE

A "Quick Reference" epidemiology summary table is online at: <u>http://www.adph.org/environmental/lofgrentable.htm</u>

A more detailed epidemiology summary table from FDA is online at: http://vm.cfsan.fda.gov/~mow/app2.html