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CHAPTER

Food Microbiology: A Review

INTRODUCTION

Microbiology is defined as the science that deals with the study of micro-organisms, including algae, bacteria, fungi, protozoa, and viruses. Specifically, bacteria are the most abundant of all organisms, they are unicellular, are relatively small ranging in size from 0.5 to 5.0 µm, and for the most part they reproduce asexually. Although there are bacterial species capable of causing human illness (pathogens) and food spoilage, there are also beneficial species that are essential to good health and the environment (examples include: synthesise vitamins, digest plant cellulose, fixing nitrogen in plant roots, etc.).

Every bacterial species have specific nutritional requirements, temperature, humidity, etc. for energy generation and cellular biosynthesis. The bacterial cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation and under favourable conditions, a growing bacterial population doubles at regular intervals ranging from about 15 min to 1 h. This means that if we start with 1,000 cells with a generation time of 30 min then after an hour we end with 4,000 cells.

Bacteria are also known as prokaryotes because they do not possess nuclei; i.e. their chromosome is composed of a single closed double-stranded DNA circle. Structurally, a prokaryotic cell has three architectural regions: appendages (attachments to the cell surface) in the form of flagella and pili (or fimbriae); a cell envelope consisting of a capsule, cell wall and plasma or inner membrane, and a cytoplasmic region that contains the cell genome (DNA), ribosomes and various sorts of inclusions.

Food microbiology encompasses the study of micro-organisms which have both beneficial and deleterious effects on the quality and safety of raw and processed meat, poultry, and egg products. Food microbiology focuses on the general biology of the micro-organisms that are found in foods including: their growth characteristics, identification, and pathogenesis. Specifically, areas of interest which concern food microbiology are: food poisoning, food spoilage, food preservation, and food legislation. Pathogens in product, or harmful micro-organisms, result in major public health problems in the United States as well as worldwide and is the leading causes of illnesses and death.

MICRO-ORGANISMS AND FOOD

The micro-organisms occurring on and/or in foods are from a practical point of view divided into three groups: molds, yeast and bacteria. Molds are generally concerned in the spoilage of foods; their use in the food industry is limited (e.g. mold ripened cheese). Yeasts are the most widely used micro-organisms in the food industry due to their ability to ferment sugars to ethanol and carbon dioxide. Some types of yeast, such as baker's yeasts are grown industrially, and some may be used as protein sources, mainly in animal feed.

Bacteria important in food microbiology may be divided into groups according to the product of fermentation, e.g. lactic acid bacteria, acetic acid bacteria, propionic acid bacteria. Bearing in mind the food constituent attacked (used as food for micro-organisms), proteolytic, saccharolytic and lipolytic bacteria may be distinguished.

Their systematic classification is based primarily on morphological and physiological properties (e.g. aerobic and anaerobic bacteria, gas forming bacteria, etc.). Lactic acid bacteria are widely used in the dairy industry, and acetic acid bacteria in vinegar production. Many bacteria are known as micro-organisms that cause spoilage and some are pathogens (e.g. *Salmonellae*, *Staphylococci*, etc.).

Both foods of plant and animal origin normally carry a microflora on the surface of their parts. Animals also have an intestinal microflora. Both animals and plant may also become contaminated from outside sources. The inner, healthy tissues of plants and animals, however, have been reported to contain few living micro-organisms, or none.

The fruit or vegetable is harvested, milk is drawn, fish and other products are obtained from natural waters, and animals are collected and slaughtered—all carrying their usual microflora. After initial handling, further contamination begins and it continues while the product is being processed and prepared.

Foods may be contaminated by each other and by pieces of equipment with which they come in contact. Air, dust, water and ingredients may add their quota of contaminants. Whenever, food is handled personally by human beings, there is always the possibility of addition of human pathogens.

In the framework of this chapter a brief overview will be given about micro-organisms that play an important role in production, storage and consumption of foods. Their occurrence, characteristics used for identification, conditions of growth and eventual industrial use will also be treated.

Bacteria in Food Production

Bacteria are used to make a wide range of food products. The most important bacteria in food manufacturing are *Lactobacillus* species, also referred to as lactic bacteria.

Dairy industry: It would be impossible to make cheese without a starter culture. As the culture grows in the milk, it converts the sugar lactose into lactic acid, which ensures the correct level of acidity and gives the cheese its moisture. As the cheese ripens, the culture gives it a balanced aroma, taste, texture. It is also responsible for the 'holes' in cheeses such as Emmenthal. Choosing the right mixture of culture is essential for a high-quality cheese.

In yogurt and other fermented milk products, the culture is responsible for the taste and texture of the final product. Depending on the acidity, the product will have either a mild or strong taste, and the viscosity depends on the quantity of polysaccharides—chains of sugar molecules—that are produced.

In recent years, probiotic cultures have become popular in dairy products because of their health benefits. These cultures are all very carefully selected strains, and there is good evidence that they help improve digestion, safeguard the immune system, and keep the body's intestinal flora in balance.

Meat industry: Meat starter cultures are used to make dried, fermented products such as salami, pepperoni, chorizo and dried ham. Lactic bacteria develop the flavour and colour of the products. In addition, a wide variety of molds are used to ripen the surface of sausages, preserving the natural quality of the product and controlling the development of flavour.

Wine industry: Yeasts are responsible for the fermentation process which produces alcohol in wine. However, lactic bacteria also play an important role, as they convert the unstable malic acid that is naturally present in wine into the stable lactic acid. This conversion gives the stability that is characteristic of high-quality wines that improve on storage.

Healthfood industry: Lactic bacteria are used in many different tablets and capsules sold as supplements in the healthfood industry. Our hectic modern lifestyles often lead to an imbalance in the intestinal flora; travel and medical treatment are two of the major culprits. By taking supplements containing lactic bacteria, this balance can be restored, improving the quality of life.

Yeasts in Food Production

Yeasts have two main uses in food production: baking and making alcoholic beverages. They have been used in this way since ancient times—there is evidence that ancient Egyptians used yeast in breadmaking, and we have been making fermented drinks like beer and wine for millennia.

Baking: Baked goods like bread rise because of the presence of yeast as a raising, or leavening, agent. The most common yeast used in breadmaking is *Saccharomyces cerevisiae*. It feeds on the sugars present in the bread dough, producing the gas carbon dioxide. This forms bubbles within the dough, causing it to expand. Other ingredients in the mixture have an effect on the speed of the fermentation—sugar and eggs speed it up; fats and salt slow it down.

Brewing: Several different yeasts are used in brewing beer, where they ferment the sugars present in malted barley to produce alcohol. One of the most common is *Saccharomyces cerevisiae*, the same strain used in breadmaking; this is used to make ale-type beers and is known as a top-fermenting yeast as it forms a foam on the top of the brew. Bottom-fermenting yeasts, such as *Saccharomyces pastorianus*, are more commonly used to make lagers. They ferment more of the sugars in the mixture than top-fermenting yeasts, giving a cleaner taste.

Winemaking: The alcohol in wine is formed by the fermentation of the sugars in grape juice, with carbon dioxide as a by-product. Yeast is naturally present on grapeskins, and this alone can be sufficient for the fermentation of sugars to alcohol to occur. A pure yeast culture, most often *Saccharomyces cerevisiae*, is usually added to ensure the fermentation is reliable. Sparkling wine is made by adding further yeast to the wine when it is bottled. The carbon dioxide formed in this second fermentation is trapped as bubbles.

Molds in Food Production

Molds are essential components of several food products, such as some cheeses, sausages and soya sauce.

Cheese making: Three main types of cheese rely on molds for their characteristic properties: blue cheese, soft ripened cheese and rind-washed cheese.

To make blue cheese, the cheese is treated with a mold, usually *Penicillium roqueforti*, while it is still in the loosely pressed curd form. As the cheese matures, the mold grows, creating blue veins within it which gives the cheese its characteristic flavour. Examples include—Stilton, Roquefort and Gorgonzola.

Soft ripened cheese such as Brie and Camembert are made by allowing *Penicillium camemberti* to grow on the outside of the cheese, which causes them to age from the outside in. The mold forms a soft white crust, and the interior becomes runny with a strong flavour.

Rind washed cheeses like Limburger also ripen inwards, but here, as the name suggests, they are washed with brine and other ingredients like beer and wine which contain mold. This also makes them attractive to bacteria, which add to the flavour.

Meat fermentation: A wide variety of molds (i.e. *Penicillium chrysogenum* and *Penicillium nalgiovense*) are used to ripen surfaces of sausages. The mold cultures plays a role in aroma formation and improve the texture of the sausages. They also contribute to shortening of the ripening period and preserving the natural quality and in that way expanding the shelf-life of the meat product.

Inoculations of sausages with molds were traditionally done with the indigenous flora of the slaughters, the so called house flora.

Soya sauce: Traditional soya sauce is made by mixing soyabeans and other grains with a mold—either *Aspergillus oryzae* or *Aspergillus sojae*—and yeast. Historically, this would have been left to ferment in the sun, but nowadays it is mostly made under industrial conditions. The key flavour ingredients formed in this process are salts of the amino acid, glutamic acid, notably monosodium glutamate.

FOOD SPOILAGE AND PRESERVATION

Food starts to deteriorate or spoil from the time that it is harvested or slaughtered. The process commences with enzyme action which occurs as the cells in the food die. It continues with action by a range of spoilage bacteria and, in some foods, molds and yeasts. The food will develop off flavours, odours and a breakdown in texture, providing recognisable signs that the food is no longer fit to eat.

In order to slow down the spoilage process and thus keep foods for a longer period, a wide range of preservation methods are applied to foods. Most methods involve the removal or control of factors which affect bacterial growth are given below:

- Temperature control—the use of low or high temperatures

- Moisture control—dehydration
- Use of chemicals
- Physical methods.

In general, more than one method of preservation will be employed to achieve an acceptable shelf-life for products. A combination, for example, of some form of heat processing combined with the use of a chemical preservative, will often produce a more durable product than if a single preservation method were used.

The use of different preservation methods will be influenced not only by the shelf-life required but also by the demand for quality in terms of taste, smell, appearance, nutritional value, etc. of the food as some methods may significantly affect these aspects.

Low Temperatures

The use of low temperatures slows down the speed of enzyme reaction and also prevents the growth of most pathogens and food spoilage bacteria.

Preservation methods using low temperatures include:

- Refrigeration—storage between 0 and 5°C.
- Freezing—storage at 18°C; there are a variety of commercial techniques to freeze foods which include fluidized-bed freezing, air-blast freezing, plate freezing, cryogenic freezing and the 'pellofreeze' system.

High Temperatures

Heat is used to destroy both pathogenic and spoilage bacteria; some heat processes will also destroy spores.

Preservation methods in this category include:

- Pasteurisation—time and temperature combination to destroy active pathogens and some spoilage bacteria in the product, e.g. for milk 72°C for 15 sec.
- Sterilisation—destruction of all micro-organisms using temperatures above 100°C.
- Ultra-heat treated (UHT) used for some products to destroy micro-organisms without causing significant change to flavour, relying upon high temperature for a very short time.
- Cooking—usually used to improve palatability rather than to improve storage quality.
- Ohmic heating involves use of high-voltage electric current through foods to destroy micro-organisms.
- Canning—involves use of heat to appropriate temperature and for a prescribed time to destroy micro-organisms, including *Clostridium botulinum* spores. Process also includes removal of oxygen and hermetic sealing of containers to avoid post-process contamination.

One of the largest hurdles the food industry has to overcome is food preservation and spoilage. Food spoilage accounts for a significant loss of material, wasting energy and water. Multiple preventive techniques are used such as packaging, natural ingredients, heat treatment, dehydration, acidity, air conditions, decreasing water activity, irradiation, refrigerator or freezer temperatures and addition of food additives to decrease osmotic pressure.

Bacterial cells, molds, and yeast may grow and spoil a food substance, allowing it to be unappealing to the consumer. For example, yogurt is manufactured to a pH of 4.6, which will inhibit growth of many other bacteria but fungi contamination of yeast and molds may occur due to addition of fruit. The doming of the lid after temperature abuse indicates gas formation by yeast fermentation. Mold is dependent on multiple variables for growth including oxygen availability, high moisture, temperature, pH and nutrients. Storing foods at refrigerator temperature will not inhibit growth but may prolong the shelf-life of a product as will packaging which is not oxygen permeable or replacing the oxygen with a different gas, such as carbon dioxide.

Inhibitors are chemicals or substances added to another substance to slowdown or prevent a reaction or change. Inhibitors which repress growth of fungi and bacteria may also be added to a food product to increase shelf-life. The amount of inhibitor, also known as a preservative or food additive, is dependent on the food product and the bacteria or fungi whose growth is needed to be repressed.

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FOOD SAFETY

Food safety is a scientific discipline describing handling, preparation, and storage of food in ways that prevent foodborne illness. This includes a number of routines that should be followed to avoid potentially severe health hazards.

Food can transmit disease from person-to-person as well as serve as a growth medium for bacteria that can cause food poisoning. In developed countries there are intricate standards for food preparation, whereas, in lesser developed countries the main issue is simply the availability of adequate safe water, which is usually a critical item. In theory food poisoning is 100% preventable. The five key principles of food hygiene, according to WHO, are:

1. Prevent contaminating food with pathogens spreading from people, pets, and pests.
2. Separate raw and cooked foods to prevent contaminating the cooked foods.
3. Cook foods for the appropriate length of time and at the appropriate temperature to kill pathogens.
4. Store food at the proper temperature.
5. Do use safe water and cooked materials.

FERMENTATION

Fermentation is one of the oldest methods of processing food into a form that is suitable for preservation. For example, making cheese is a good way of preserving milk. Wine is an excellent means for preserving grapes. Kim chi is a perfect vehicle for preserving and enhancing the flavour of the humble cabbage.

Fermentation probably began from baser motives, however. In the hot climates of the Middle East fruit rapidly deteriorates. Many fruits ferment naturally producing acids and alcohol. People eating this fermenting fruit would have noticed a different flavour from the build up of acid and perhaps a slight effect from the small amounts of alcohol present. This would have been enough to set curious minds on the path to discovering the benefits of controlled fermentation to produce alcoholic drinks.

Reliable reports show that alcohol was being produced from fruit in Babylon almost 7000 years ago. The same peoples were among the first to ferment milk as well.

Benefits of Fermentation

One of the most important reasons to ferment foods is that it is a cheap and energy efficient form of preservation.

Fermentation makes food products more interesting by intensifying the flavour through the conversion of sugars into acids.

Fermenting makes grains more digestible. For example, porridge that has been fermented (as is common in Africa and even in Wales) hydrolises starch into shorter chains of glucose and dextrose.

Fermented products often contain higher levels of vitamins (particularly thiamine, nicotinic acid, biotin and riboflavin) and proteins. Examples are the Mexican drink called pulque, the Indian fermented bread called idli, sorghum beer from Southern Africa and palm wine from West Africa.

Some fermented products have meat-like flavours and odours which is important for cultures where meat is scarce (e.g. soya and fish sauces). South East Asian countries had an excess of fish products and a paucity of meat. It made sense to make something that reminded the senses of meat.

Fermentation can reduce naturally occurring toxins in some foods thus rendering them safe to eat (e.g. Cassava which is widely eaten in Africa has semi-dangerous levels of cyanide. By fermenting cassava to produce Kawal the cyanide is rendered harmless.)

Fermented foods often contain a higher level of convertible energy than non-fermented foods of the same weight.

There are medical advantages associated with a constant intake of some fermented foods (koumiss is used in Russia to treat tuberculosis).

Fermentation Process

There is not common agreement on a definition of fermentation. It all depends on who is defining it and what their perspective on the process is. A food aid person might define it as a relatively low-cost means of using bacteria, yeasts and molds in preserving food and enhancing its flavour and nutritional value.

A food technologist, however, might see it in a different way. To them, fermentation is a process by which bacteria, yeasts and molds convert sugars and carbohydrates into less complex products such as carbon dioxide and alcohol.

A key point about fermentation from a chemical perspective is that relatively complex organic compounds are split into simpler chemicals.

We exclude from the definition processes such as drying, canning, freezing and pasteurising for two reasons. The first is that they are all about excluding bacteria, yeasts and molds. Secondly, the equipment required is usually (with the exception of drying) high cost.

Everyone knows what bacteria are, but not everyone realises that there are 'good' bacteria as well as 'bad' bacteria. Some of the fermentation processes actually encourage the presence of bacteria to break- down inedible or harmful food products and render them safe for consumption. A good example is the introduction of bacteria into milk products to produce yogurt.

Yeasts and molds are less well understood by the general community. Both are fungi and are by nature parasites. They must feed off other substances such as soil, wood, leaves or fruit. Yeasts chomp away at the barley malt to produce the alcohol for beer. Molds are injected into cheeses to produce the fabulous flavour of a gorgonzola or stilton.

A fundamental chemical underpinning of fermentation is that an acidic environment is created. Many harmful organisms cannot exist in an acidic solution.

Bacterial fermentation

We all know about bacteria spoiling our food. Just leave a glass of milk in the sun for a couple of hours and those tiny microbes will have had more fun.

But bacteria can be harnessed for good as well. Bacteria added to milk in the presence of rennet starts the process of cheese production. Bacteria added to chopped cabbage produces the acidic environment required for the creation of sauerkraut in Europe, kim chee in Korea. A similar process is used for cucumber pickles in the United States, khalpi and gundruk in Nepal, hum choy in China and torshi khiar in Egypt.

Bacteria are either single or multi-cell organisms that are found just about everywhere in nature. Often, but not always, they contribute to food spoilage. They are voracious eaters. They do this by sending enzymes out through their cell walls on raiding parties into the surrounding environment. These enzymes attack food sources and break them down into particles that can be absorbed by the cell through osmosis.

Some bacteria are particularly fond of carbohydrates found in dairy products and can rapidly convert them into lactic acid. Far fewer are very keen on the sugars in fruit and break them down to produce acetic acid. The reason that not so many bacteria are successful at fermenting fruits is that growing fruits are usually high in acid—the nemesis of many bacteria.

Not so with vegetables, however. Most vegetables are low acid and very susceptible to bacterial fermentation. Therefore, the fermentation of vegetables into a high acid environment (sauerkraut, pickles, kim chee) is a perfect way of preserving them. The acids produced by fermentation are the slightly sour taste that can be recognised in many of our favourite foods including buttermilk, yogurt, sauerkraut, pickles and even olives. As the acid levels increase, harmful bacteria are killed off.

Lactic acid bacteria go to work on carbohydrates found in flour, grain, dairy products and vegetables to produce an acidic environment that is both suitable for the preservation of the food and for changing the nature of the flavour of the food.

Thus, lactic acid bacteria are responsible for the highly desirable sour taste in sourdough bread. They are responsible for the acidic nature of sauerkraut. They are even responsible for nullifying the harmful cyanide substances in cassava enabling the production of the widely eaten gari and fufu in Africa. In this case the lactic acid bacteria turn the cyanide compounds into cyanic gases which then escape from the fermenting food, thus rendering it harmless.

By producing the acidic environment they cause other, harmful bacteria to die, thus making the food safe for storage and subsequent consumption.

In some cases the process is quite complex. For example, the production of sauerkraut requires the presence of at least *three different types of* lactic acid bacteria as well as salt and pressure to release the sugary juices from the cabbage.