FOOD MICROBIOLOGY

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Contents

Summary

Foods, either of animal or plant origin, carry on their surfaces a natural microflora determined primarily by the type of plant or animal and environmental conditions such as the microflora of ambient air and soil. The inner, healthy tissue of plants does not contain living microorganisms. Animals, in addition to surface microflora, also have an intestinal microflora.

The number and types of microorganisms may considerably increase during handling and processing of foods. Foods may be contaminated by each other during storage and processing by equipment used in food manufacture and by humans involved in handling. From the practical viewpoint, the thousands of genera and species of microorganisms occurring in foods may be divided into three groups: molds, yeasts, and bacteria.

On one hand, molds are organisms generally causing spoilage of foods. On the other hand, molds are used in production of some types of cheese and fermented foods and in industrial production of, for example,citric acid or enzyme preparations.

Yeasts are known to non-specialists primarily as useful microorganisms, although spoilage of several foods e.g. fruits may be caused by these organisms. Production of beer, wine and other alcoholic beverages is based on fermentative activity of yeasts. Yeast biomass may be used for feed purposes and yeast protein preparations, and autolysates are also used as food ingredients.

Bacteria important in food may be divided according to the main product of fermentation. Consequently lactic acid bacteria (used in the dairy industry), acetic acid bacteria (utilized in vinegar production), propionic acid bacteria (being active in some types of cheese), and pigmented bacteria (generally causing spoilage of foods), are distinguished. On the other hand, the basis of classification may be the main food component (substrate) attacked by the microorganism (proteolytic, lipolytic, and saccharolytic bacteria). A great range of bacteria play a role in food spoilage and occurrence of human or animal pathogens is also possible.The characteristics of most important molds, yeasts and bacteria are summarized in this contribution.

Although several factors influence the frequency and rate of spoilage of foods, the decisive role concerning spoilage belongs to microorganisms. Thus, the prevention of spoilage and preservation of foods needs exclusion of activity of microorganisms. Killing of microorganisms by high temperature treatment is the most effective means of preservation.

Reduction of water content (available water or water activity), e.g. drying and use of low temperatures (chilling, freezing) stops the growth and activity of microorganisms, but most microbes survive, so storability is limited. Chemical preservatives, including antibiotics, may be used to inhibit the growth of microorganisms or kill them.

Although the safety of permitted preservatives is carefully controlled, there is a general tendency to reduce the use of chemicals in food preservation. Use of ionizing radiations and high mechanical pressure (a recent method of preservation of foods) are also briefly discussed.

The relatively high frequence of outbreaks of food-borne diarrheal diseases, even in industrially developed countries, has resulted in growing interest and intensive research in the field of food pathogens. The microorganisms causing food infection and poisoning (such as *Staphylococci, Salmonellae, Listeria, Campylobacter*) are also overviewed. Finally methods used in food microbiology are considered.

1. Introduction

Since the investigations of Pasteur in the ninetenth century, resulting in the discovery of microorganisms, it is widely known that all foods of plant and animal origin normally carry a microflora on their surfaces. Animals also have an intestinal microflora. Both animals and plants may become contaminated from outside sources. The inner, healthy tissues of plants and animals, however, have been reported to contain few living microorganisms, or none.

During handling and processing further contamination begins. Foods may be contaminated by each other and by all pieces of equipment with which they come in contact, and humans involved in handling and processing are potential sources of microorganisms. Air, dust, water, and ingredients may add their quota of contaminants. (The sources of microbial contamination of foods are treated in detail in *Spoilage and Preservation of Food).*

The aim of food microbiology is to give an overview of microorganisms important in foods, to outline briefly their characteristics useful in identification, to discuss their role in spoilage of foods and in application in food production and their importance regarding food safety including food-borne diseases.

This Topic level contribution summarizes the basic knowledge concerning the importance of food microorganisms in spoilage, preservation, and manufacture of foods, and public health. These subjects are discussed in more details in the six Articles belonging to this topic.

2. Microorganisms Important in Food.

Bearing in mind that according to systematic classification, thousands of genera, strains and species may be distinguished (described in large manuals) here three arbitrary groups, usually created by food microbiologists on the basis of practical aspects, will be discussed namely: molds, yeasts and bacteria.

2.1. Molds

2.1.1. General

The term ***mold*** is applied to certain multicellular, filamentous fungi whose growth on foods is usually readily recognized by its fuzzy or cottony appearance. Molds are known on one hand as organisms generally causing spoilage of foods. On the other hand, some molds are used in manufacture of different foods and are ingredients of some types of cheese. Molds are also used for production of several enzymes and antibiotics.

Macroscopically the mold consists of a mass of branching, interwined filaments called *hyphae (*singular hypha), and the whole mass of these hyphae is known as the *mycelium.* Hyphae may be classed as *vegetative* or *fertile* based on their biological function. The vegetative hyphae or growing hyphae are concerned with the nutrition of the mold and the fertile ones with the production of reproductive parts. With microscopic study further details of molds may be recognized. The reproductive parts or structures of molds are the *spores*, which are mainly asexual. Such spores are produced in large numbers and are readily spread by air to alight and start new mold plants where conditions are favorable.

Physiological characteristics. In general, molds require less moisture than bacteria and yeasts. The minimum water activity for spore germination has been found to be as low as 0.62 for some molds and as high as 0.93 for others. Each mold has an optimum of water activity and a range of water activity for growth. Most molds grow well at ordinary room temperatures and are classified as *mesophilic*. The optimum for most molds is between 25 and 30 oC. Nevertheless it should be noted that some of the molds grow fairly well at temperatures around freezing or just above, and some can grow slowly at sub-zero temperatures.Molds require free oxygen for growth, which is why molds grow on the surface of contaminated food. Most molds grow over a wide range of pH, but some are favored by acid foods such as the majority of fruits.

2.1.2. Molds Occurring in Foods

It is beyond the scope of this contribution to give a full overview of molds occurring in foods, so in the following only genera of industrial importance will be briefly mentioned.

Mucors are involved in the spoilage of some foods and in manufacture of others e.g. oriental fermented foods. *Rhizopus nigricans,* sometimes called ’bread mold’, is a very common mold occurring in foods. It is involved in the spoilage of many foods such as berries, fruits, vegetables, bread, etc.

Members of genus ***Aspergillus*** are very widespread. Many are involved in the spoilage of foods and some are useful in preparation of fermented foods. Many groups and hundreds of *Aspergillus* species are known. *Aspergillus niger* is the leading species of importance for food microbiologists. Selected strains are used for commercial production of citric and gluconic acids.

Penicillium is another widespread genus important in foods. *Penicillium expansum*, a green spored mold, causes soft rot of fruits. *Penicillium camemberti* with grayish conidia, is useful in the ripening of Camembert cheese, and *Penicillium roqueforti* is used in ripening of blue cheeses.

Species of the genera *Bothrytis, Alternaria,* and *Neurospora (Monilia)* grow on various foods.

2.2. Yeasts

2.2.1. General

Although the term ***yeast*** is used extensively in the scientific literature, it has been difficult to state a precise definition of yeasts based on common morphological, physiological or other characteristic properties. If we omit the numerous exceptions, it may be stated for most yeasts that they are *single-celled, colorless, and bud forming; the cell shape is round or oval, and they are able to grow in the absence of air.*

Most yeasts reproduce asexually by budding. The term budding means a process in which some of the protoplasm bulges out through the cell wall, the bulge grows in size and finally walls grow around it to form a new yeast cell. Some yeasts (true yeasts) reproduce sexually through ascospores).

Yeasts are divided into two groups based on the type of growth in or on liquid media. Some yeast species form a film or scum on the surface of liquid. These are called film-yeasts or oxidative yeasts. Others grow throughout the liquid medium.

The lower limits of water activity for ordinary yeasts tested thus far range from 0.88 to 0.94. The optimum range of temperature for growth of most yeasts is around 25 to 30 ^{o}C and the optimal range of acidity is pH=4 to 4.5.

In general, sugars are the best food for yeasts. All fermentative yeasts are able to ferment glucose to produce ethanol and carbon dioxide. This fermentation process is used in practice for producing beer, wine, and industrial alcohol, and carbon dioxide produced by baker's yeasts accomplishes the leavening of bread. Yeasts can utilize both simple inorganic (ammonia and in some species also nitrate) and organic (urea) compounds as a nitrogen source, but also amino acids, peptides and polypeptides. In addition to sugars (sources of carbon, oxygen and hydrogen) and N-containing compounds, the yeasts need several minor biologically important compounds commonly named growth factors.

2.2.2. Classification,Important Genera of Yeasts and Their Industrial Use.

According to botanical classification yeasts belong to the division Fungi and the yeasts found in food are divided into classes: *Ascomycetes* and *Fungi imperfecti.* According to taxonomic characteristics Orders, Families, and Genera are distinguished.

One of the most important is the genus *Saccharomyces. Saccharomyces cerevisiae* is the leading species used in the baking industry for leavening bread, production of beer and wine, production of ethanol and several other products used in the food industry. *Saccharomyces cerevisiae* var. *ellipsoideus* is known as wine yeast because this is a high-alcohol-yielding variety. It is also used in industrial ethanol production with fermentation technology.

Among true yeasts *(Fungi imperfecti*) we should mention film yeasts such as genus *Candida* and *Mycoderma*, which grows on wine, beer, cheese, pickles, sauerkraut, and other fermented products and take part in their spoilage.

The commercial production of yeast started in the second half of the nineteenth century. Strains of *Saccharomyces cerevisiae* to be used in manufacture of baker's yeast are grown on a medium of molasses and mineral salts. During growth of the yeast the medium is aerated at a rapid rate. The yeast is centrifuged out in the form of „cream", and this is put through a filter press to remove excess liquid. The mass of yeast is made into cakes of different size after incorporation of small amounts of vegetable oil.

In modern wine technology carefully selected yeast species are widely added to crushed grapes. These strains are varieties of *Saccharomyces ellipsoideus*, a high-alcohol-yielding strain, and they contribute to the specific flavor of famous wine types. For champagne production specific yeasts, tolerant of high alcohol content and carbon dioxide pressure are selected.

The incidental consumption of microbes by humans in fermented foods and that of distiller's and brewer's spent grains by domestic animals are long-established practises. However a conscious effort to grow microorganisms for human diet started in the twentieth century. Due to their high protein and vitamin content, yeasts may be used for food purposes. At present considerable amounts of yeast are produced for feed and food purposes. Yeasts produced directly for such purposes are termed *primary* and those recovered as by-products of a fermentation process, e.g. brewing, are called *secondary.*

Protein concentrates and isolates, often called *single-cell protein* may be produced and used for protein enrichment of low-protein foods. Protein is rich in lysine but poor in sulfur-containing amino acids, particularly methionine. Another problem is connected with the relatively high nucleic acid content of preparations. For example, for humans an upper limit of daily intake of 2g nucleic acids has been generally accepted (because of potential build-up of uric acid). Several processes have been developed for reduction of nucleic acid content in yeast protein preparations.

Yeasts can synthetize many of the vitamins and some provitamins. Concerning the concentration level of vitamins in yeast biomass, it should be mentioned that yeasts can absorb thiamine, niacin, biotin, and, to lesser extent, pyridoxine, inositol, and other vitamins. Therefore the level of these vitamins in the substrate in which the yeasts are grown is a factor in determining the vitamin content of the yeast cells.

2.3. Bacteria

2.3.1. General

Bacteria are minute, single-celled organisms of variable shape and activity. There are thousands of different types everywhere in air, in soil, water, the surface of plants and animals, and consequently in foods, and in the digestive tract of animals and humans. Fortunately, the majority of bacteria perform useful functions in the environment and also in some branches of the food industry, such as production of some dairy products, bakery products, vinegar or antibiotics for the pharmaceutical industry. Only a very small proportion of the total bacterial population are dangerous because they can cause disease in man and animals. Bacteria involved in food-borne diseases are described in *Food-borne Pathogens* and there is also an Article on *Lactic Acid Bacteria*, so they will be only treated indirectly in this article.

Morphological characteristics. The cells of these non-filamentous and non-photosynthetic organisms are relatively simple and quite small. Cocci have a diameter of about 0.5 to 1.0 micron, and rods rarely exceed 1 micron in width but may attain a length of 20 microns.

Bacteria of the genus *Bacillus* and *Clostridium* form endospores, but rod forms do not, and nor do the cocci encountered in foods. Spores of different bacterial species or even different strains vary widely in their resistance to heat and other adverse conditions. In general, however, bacterial spores are considerably more resistant to heat, chemicals, and other destructive agents than the vegetative cells.

Growth and multiplication. Bacteria multiply by simple division into two, and under suitable conditions of environment and temperatures this occurs every 20 to 30 minutes. Thus one cell could become over two million cells in 7 hours and 7000 million cells after 12 hours continuous growth. When each cell has grown to its maximum size, a constriction appears at both sides of the center axis, the outside membrane or envelope of the cells grow inwards and forms a division which finally splits, releasing two new twin cells.

The spores produced by certain bacteria, under suitable conditions, can germinate into actively growing cells again..

Among factors influencing bacterial growth in food, moisture, temperature, acidity (pH), oxidation-reduction potential (presence or absence of oxygen), and presence of inhibitory substances, are the most important. Each bacterium has a definite range of food requirements. As carbon and energy source mostly carbohydrates (sugars) are utilized by bacteria. Some can use a variety of carbohydrates, and others only one or two. Several bacteria can utilize organic compounds, others only carbohydrates. The nitrogen requirements of bacteria may be satisfied by simple compounds such as ammonia or nitrates or, for example, in the case of lactic acid bacteria, more complex compounds like amino acids, peptides, or proteins may be utilized. Bacteria also vary in their need for vitamins and or accessory growth factors.

Each bacterium has an ***optimum temperature***, at which it grows best, a ***minimum temperature***, which is the lowest one at which growth can occur, and a ***maximum temperature*** at which cells can multiply. Based on the optimal temperature, bacteria are classified as ***psychrophilic*** (that grow well at refrigeration temperatures), ***mesophilic*** (optimum temperature 20 to 45 ^{o}C) and t***hermophilic*** (over 45 ^{o}C).

Many bacteria require oxygen to live actively (***aerobic bacteria***), but others can multiply in the absence of oxygen (***anaerobic bacteria***).

When the available nutrient in food (or other medium) has been exhausted or the waste products of growth have made the environment unsuitable, for example, by lowering pH, growth ceases and the cell dies. The length of life of a bacterial cell varies according to its food or medium and according to the type of microorganism. The spores can survive for long periods of time under adverse conditions.

Bacteria can live and multiply in many foodstuffs. Meats and poultry are good examples—whether raw or cooked they are excellent media for bacterial growth. The same is valid for milk and egg products, pies, stews, and gravies when stored without refrigeration.

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Biographical Sketch

Radomir Lasztity D.Sc. is a Professor of the Department of Biochemistry and Food Technology at Budapest University of Technology and Economics. He was born in 1929 in Deszk, Hungary. He received his M.Sc. degree in Chemical Engineering in 1951 and his D.Sc. degree in Chemical Science in 1968. He is honorary president of the International Association for Cereal Science and Technology (ICC) and deputy technical director. He was acting chairman of the Codex Committee on Methods of Analysis and Sampling of the FAO/WHO Food Standard Program in the period 1975 to 1988. Dr Lasztity is a member of the Food Chemistry Division of the Federation of European Chemical Societies., and a member of the editorial boards of several international scientific journals. Among other awards he has received the Bailey and Schweitzer Medal of the ICC, the State Prize of the Hungarian Republic, and the Golden Medal of the Czech Academy of Sciences. His main research activities are chemistry and biochemistry of food proteins, food analysis and food quality control. He has published more than 800 articles in Hungarian and overseas journals. He is the author/editor of more than twenty books and textbooks [*Chemistry of cereal proteins(1984, 2nd ed. 1996), Amino Acid Composition and Biological Value of Cereal Proteins (1985), Cereal Chemistry (1999), Use of Yeast Biomass in Food Production (1991), Gluten Proteins (1987)*].