# FOOD MICROBIAL ECOLOGY

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## Contents



## Summary

Microbial ecology is the study of microorganisms in their proper environment and their interactions with it. Microbial ecology can give us answers about our origin, our place in the earth ecosystem as well as on our connection to the great diversity of all other organisms. In this vein, studying microbial ecology questions should help to explain the role of microbes in the environment, in food production, in bioengineering and chemicals items and as result will improve our lives.

There is a plethora of microorganisms on our planet, most microorganisms remain unknown. It is estimated that we have knowledge only of 1% of the microbial species on Earth. Multiple studies in intestinal ecology have been greatly hampered by the inaccuracy and limitations of culture methods. Many bacteria are difficult to culture or are unculturable, and often media are not truly specific or are too selective for certain bacteria. Furthermore it is impossible to study and compare complete ecosystems, as they exist in the human body, by culturing methods. Molecular tools introduced in microbial ecology made it possible to study the composition of the microecosystems in a different way, which is not dependent on culture techniques. If we can gain a better overall understanding of microbial ecosystems and communities, then we will have a better foundation and a profound understanding of our world microbial ecology in health and disease. It is thought that at least 500 species comprising up to $10^{12}$ bacteria are harboring the healthy human intestinal tract. Moreover, it is important to make thorough considerations about the specific environment in which bacterial populations are isolated as this environment seems to change considerably under the influence of different factors.

It is then conceivable that microbes are found in every environment such as, air, water, soil and can be spread to the food commodities. The microbial ecology of food commodities is concerned with the food microbiology and ecosystem.

Specifically, it describes the natural microbial flora and the prevalence of pathogens in the different foods. Any microbial modification during processing, transport and storage stages should be critical to the food quality. The relation of food commodities with foodborne illness, and measures to control pathogens and limit spoilage is involved. It is not neglectable also the beneficial role and transformations caused by some bacteria in food commodities.

## 1. The Scope of Microbial Ecology

Microbiologists have found microbes living just about everywhere; in the soil (Berkeley et al. 1979), water (Bezirtzoglou et al. 1994, Savvaidis et al. 2003, Alexopoulos et al. 2005) air (Shiba 2009), animals (Jacobs 1962), plants (Jackson 2009), rocks (Bezirtzoglou et al. 1996) and even us (Borriello et al.1978, Bezirtzoglou et al. 1997). Microbes have been around for billions of years because they are able to adapt to the ever-changing environment.

However, many types of microbes remain unknown. It is estimated that we know less than 1% of the microbial species present on earth. Microbial Ecology is the scientific discipline of Microbiology (Campbell 1983) embedding on the study of the occurrence and significance of microbes in the environment and their interactions with each other. It compasses specialties asfood, environmental, industrial and agricultural, human, animal and clinical microbial ecology. The knowledge on microbial ecology ecosystems (Klug et al. 1984) should be a useful tool to the realistic use of microbes in environmental restoration, food and industrial production, bioengineering of useful products such as antibiotics, food supplements, as well as to the radical control of the human and animal micro floras in health and disease. Moreover, microbial ecology evolve information about the tremendous microbial diversity, their ecology, their unusual habitats, their role in bioremediation, recycling, food production , biotechnology and some clinical health disorders.Food Microbial Ecology includes the study of microorganisms colonizing and contaminating food and its environment and their implication in food-borne diseases (Ayres et al. 1968, Siliker et al. 1980, Bezirtzoglou et al. 2000, Adams et al. 2002).

## 2. Food Microbial Ecosystem

The foods we eat are rarely sterile. They carry microbial associations which composition is very different (Ayres et al. 1968, Bezirtzoglou et al. 2000, Adams et al. 2002, Montville et al. 2005). The microorganisms present originate from the natural micro flora of raw material but also, microorganisms are introduced in the course of harvesting, slaughter, processing, storage and distribution of food. In most cases, the food is consumed without objection and consequences. In same cases, microorganisms manifest their presence in 3 ways, by causing spoilage of food, by causing food borne illness or finally, they can transform food in a beneficial way; this latter is called food fermentation.

### 2.1. Food Spoilage versus Food Preservation

It is known that storage of stable nuts and grains for winter provision is done by mammals and man. With the progress in agriculture, the safe storage of surplus production is of great importance. Microbiological principles were developed empirically by people to arrest or retard the natural process of decay. Many methods have been developed for this purpose. The food preservation depended largely on water activity reduction in the form of solar drying, salting, storing in concentrated sugar solutions or smoking over a fire. The results of the exponential growth in population and the arithmetic growth in agricultural productivity would be over-population and mass starvation. At the nineteen century the development of food preservation industries started. Industrial chilling, canning and freezing permit large importation of foods from distant producers.

Nowadays, there is sufficient food to feed the world's population. Despite overall sufficiency, it is recognized that a large proportion of the population is malnourished. This is estimated to the 1/5 of the world's population. Substantial losses of food occur especially in developing countries at the pre- and post- harvest period. It has been estimated to 10% for cereals, 20% for vegetables and more than 25% for highly perishable products such as fish. It has been estimated that losses in cereals and vegetables in developing countries as 100 millions tones, would be enough to feed 300 millions people (US Agriculture Department 1997). It is clear that reduction in such losses will be important to the contribution of feeding the world's population. The agro-food sector is of major importance for the European and the international economy. The economic importance and the ubiquity of food in our life suggest safety in society as a whole, and in particular by public authorities and producers. In conclusion, there is a recognized need for simple, low-cost, effective methods for improving food storage and preservation.

### 2.2. Food Safety

Food has a long association with the transmission of disease. Special regulations concerning the food hygiene must be kept (Defigueiredo et al. 1976). The WHO (World Health Organization) at 1993 refers that: «Food borne disease is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity» (WHO 1993). The establishment of an independent European Food Authority is considered by the Commission to be the most appropriate response to the need to guarantee a high level of food safety. This Authority would be entrusted with a number of key tasks embracing independent scientific advice on all aspects relating to food safety, operation of rapid alert systems, communication and dialogue with consumers on food safety and health issues as well as networking with national agencies and scientific bodies. The European Food Safety Authority will provide the Commission with the necessary analysis. Following the Commission's Paper on food law [COM (97)176 final], and subsequent consultations, a new legal framework will be proposed. This will cover the whole of the food chain (Shapton et al. 1991), including animal feed production, establish a high level of consumer health protection and clearly attribute primary responsibility for safe food production to industry, producers and suppliers. Efficient control of contaminants and residues in foodstuffs is an essential contribution to the maintenance of a high level of consumer protection in the EU. Foodstuffs of animal and plant origin may present intrinsic hazards, due to microbiological contamination. To protect consumers from microbiological risks in food products, Community legislation sets out numerous hygienic measures (such as HACCP based principles, meat inspection etc.) called microbiological criteria (Shank 1991). Microbiological criteria (Harrigan et al. 1991) are tools that can be used in assessing the safety and quality of foods (APHA 1984, Adams 1990, Bauman 1990, Stevenson 1990, Baird-Parker 1992, Pierson et al. 1992).

### 2.3. Food Fermentation

Microbes can however play some positive role in food (Fuller 1992, Havenaar et al. 1992, Lee et al. 1995). They can effect desirable transformations in a food, changing its properties in a beneficial way. The most known example is *Lactobacillus* involved in preparation of yoghurt. This is called food fermentation. Our interest is focused on the sources of microorganisms in order to understand the ecology of contamination.

## 3. Diversity of Habitat

Microorganisms have been found in a wide range of habitats, from the coldest waters of polar regions to the boiling water of hot springs and volcanoes. They are found also at the deeper part of oceans at very high hydrostatic pressures, in the acidic wastes of mine workings or the alkaline waters of soda lakes, in black estuarine mud's or the purest waters (Campbell 1983, Klug et al. 1984). Microbes play an important role in the carbon, nitrogen and sulfur cycles (see Fig.1). Thus, they are of capital significance in the maintenance of the stability of the biosphere (Campbell 1983, Klug et al.1984).

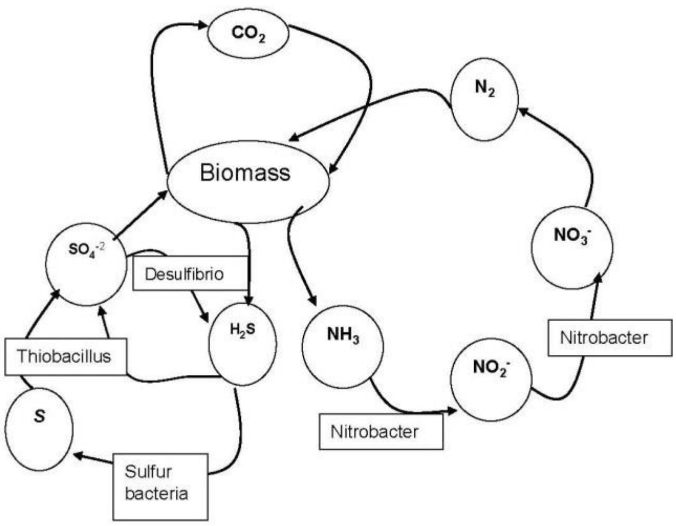


Figure 1. Micro organisms and carbon, nitrogen and sulphur cycles

They are found also on the surfaces of plants (leaves, flowers, fruits, roots) and on the surfaces and guts of animals and man (skin, intestinal flora, normal flora) where they may affect the food during manufacture of foods processing and handling

### 3.1. Microorganisms of Soil

Each soil has its own diverse flora of bacteria, fungi, protozoa and algae.
The soil is a rich reservoir of microorganisms in vegetative and spore forms. It provides strains used for the industrial production in pharmaceutical or food industries of antibiotics, enzymes, amino-acids, vitamins. The soil microorganisms (Berkeley et al. 1979, Moir 2011) participates in the recycling of organic and nitrogenous compounds (see Table 1). Finally, the soil bacteria produce resistant structures, as the endospores of *Bacillus* and *Clostridium* which can resist to desiccation and a wide range of temperature fluctuations.

### 3.2. Microorganisms in the Atmosphere

One of the most hostile environment for microorganisms is the atmosphere and this is explained by the damaging effects caused by the energy of the sun and by damaging chemical activity of the gaseous $O_2$ (oxygen). It is important to note that, Gram-negative bacteria are very sensible in air because they possess a thin layer cell wall and they are not protected in this way by radiation of the sunlight or the chemical activity of the oxygen (Shiba 2009, Sen et al. 2011).

#### 3.2.1. Airborne Bacteria and Fungi

The quantitative determination of the numbers of bacteria requires specialized sampling equipment but qualitative estimate can be obtained by exposing a Petri dish with an agar to the air for a measured period of time (see Photo 1). The bacterial flora at the air is dominated by Gram-positive rods and cocci, pigmented colonies of micrococci or corynebacteria, large white-to-cream colonies (*Bacillus*) and finally tough colonies of filamentous bacteria (*Streptomyces*) (Shiba 2009, Bezirtzoglou 2010, Sen et al. 2011).

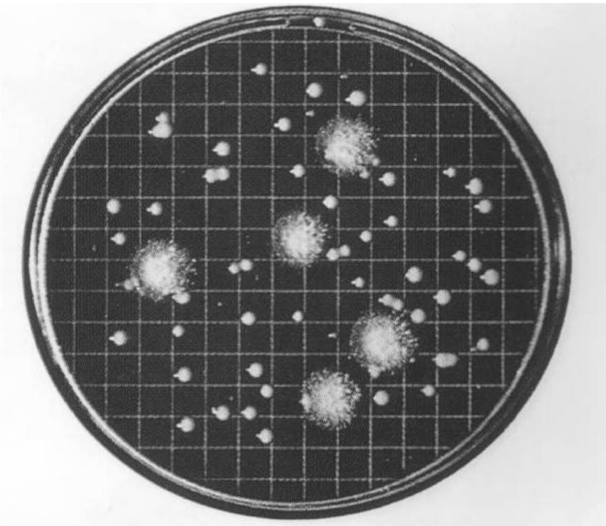

Photo 1. Qualitative estimate of microbes by exposing a Petri dish with an agar to the air

The presence of the above bacterial species is justified from the following statements: the possession of pigments protects microorganisms from damage by both visible and ultraviolet radiation of sunlight, the thick cells walls of Gram-positive bacteria protect them from desiccation and finally, endospores of *Bacillus* and Conidiospores of *Streptomyces* are resistant to the damaging effects of the air exposition (Shiba 2009, Sen et al. 2011).

The effects of radiation and desiccation are enhanced by the "open-air factor", which causes more rapid death rates of sensitive Gram-negative organisms such as *E. coli*.

During the night time, in spite of reduced light damage to the cells, microorganisms die more rapidly in open-air. It is possible that light destroys the open-air factor. We can understand that routine monitoring of air quality within a food factory or storage area is absolutely necessary (Jay et al. 1999, Bezirtzoglou 2003, Montville et al. 2005).

Bacteria have no active mechanisms for becoming airborne. They are dispersed on the dust particles, on the droplets of water during coughing and sneezing, by bursting of bubbles, by impaction of a stream of liquid onto the surface or when taking a wet stopper out of a bottle. The "Farmer's lung" disease occurs in individuals which have become allergic to the spores of Actinomycetes when exposed in the air of some farm yard. *Actinomyces* are rarely implicated in food spoilage but geosmin-producing strains of *Streptomyces* are often responsible for earthy odors and off-flavors in potable water. It is also reported that geosmin can gave earthy taints to shell fish. *Thermoactinomyces vulgaris* and *Micropolyspora faeni* are the involved species (Jay et al. 1999, Bezirtzoglou 2003, Montville et al. 2005).

The fungi become airborne as fine dry dust particles by physical disturbance and wind. The spores of *Penicillium* and *Aspergillus* are often responsible for food spoilage. *Fusarium*, produce wet table spores which are dispersed in the air into tiny droplets of water and distributed during wet weather (Jay et al. 1999, Bezirtzoglou 2003, Montville et al. 2005).

*Cladosporium* spores are released into the air, when the Relative Humidity (RH) decreases with the change from night to day and especially at the middle of the day. *Clostridium herbarum* grows well at refrigeration temperatures by forming black colonies on foods especially on chilled meat (Adams 1990). *Ballistospores* of mirror yeasts are present in highest numbers in the middle of the night when the Relative Humidity (RH) is at its highest also.

### 3.3. Microorganisms of Water

The aquatic environment represents in area and volume the largest part of the biosphere and both fresh water and the sea contain many species of microorganisms (Bezirtzoglou et al. 1994, Savvaidis et al. 2003, Alexopoulos et al.2005).

The bacteria isolated from open ocean requires salt, grow better at low temperature and are adapt generally to low concentrations of organic and nitrogenous compounds (Moir 2011). In scientific term they are called *«oligotrophic psychrophiles with a requirement* *for sodium chloride (NaCl)»*.The surfaces of fishes have a flora which reflects the above environment. Many of these bacteria can break down proteins, polysaccharides and lipids at refrigeration temperatures (0 - 7$^{o}$ C) as short as ten hours. Once these bacteria arrive to $10^{7}$ or $10^{8}$, they can be responsible of off-odors and spoilage. During the handling, fishes can be contaminated from the human bacterial flora with genus as *Enterobacteriaceae* or *Staphylococcus*, which grow at 37$^{o}$ C.

If we want to distinguish the environmental from the "handling flora", we can compare the numbers of colonies obtained by plating-out samples on a nutrient agar at 37$^{o}$C, with plates of lower concentration of organic compounds at 20$^{o}$C (Alexopoulos et al. 2011).

The seas around the coast are influenced by terrestrial and freshwater microorganisms and also by human activities, as sewage and waste products.

The shellfish grows usually in polluted waters near the coast. If these waters are contaminated with enteric microorganisms from infected people and will be concentrated by the filter feeding activities of shellfish, diseases like gastroenteritis, hepatitis or typhoid fever can occurred. In warmer seas, even unpolluted water may contain high numbers of *Vibrio parahaemolyticus*, who is responsible for outbreaks of food poisoning associated with sea foods.

Fresh waters of rivers and lakes include some aquatic species but mainly terrestrial, animal and plant sources microorganisms (Alexopoulos et al. 2006, Kirschner et al. 2009). Contamination can happen with sewage effluent containing human fecal material. These microorganisms don’t multiply usually and may be very low. If a bacterium that usually is present in large numbers in the human intestine is found, there is contamination. Such a bacterium is called an "Indicator microorganism" such as *E. coli*, fecal *Streptococci*, *C. perfringens* (Bezirtzoglou et al. 1990, 1999) and *B. fragilis*.

The fungi are also present in marine and fresh waters and they could contaminate mollusks and fish. The most frequently isolated are Ascomycetes, Basidiomycetes, Zygomycetes and Deuteromycetes.

Of the aquatic photosynthetic microorganism, the Cyanobacteria or blue-green algae (Prokaryotes) and the Dinoglagellates (Eukaryotes) can produce very toxic metabolites which may become concentrated in shellfish. Subsequently, when consumed by humans a very severe disease called "paralytic shellfish poisoning" can occurred (Jay 1998, Montville 2005).

### 3.4. Microorganisms of Animal Origin

All healthy animals, as well as the man, have a very complex microflora, well adapted to growth and survival of its host (Jacobs 1962).

Part of this flora can be transient, and reflects the immediate interaction with the usual environment.

### 3.4.1. The Skin

The surfaces of humans and animals are exposed to the air, soil and water and they could contaminate food during handling (Noble 2010). However, the surface of the skin is not favourable for microorganisms since it is usually dry, with a low pH, due to the excreted organic acids, and many microorganisms are "transient".Some microenvironments, as hair follicles, sebaceous glands, hides of skin constitute of 'microenvironments' were microorganisms grow better. In humans, the normal skin flora is composed mainly by Gram-positive microorganisms, such as *Staphylococcus*, *Corynebacterium* and *Propionibacterium*.

### 3.4.2. The Nose and Throat

The bacteria of this flora are usually harmless but may have the potential to cause disease. It is known that *Staphylococcus aureus* is carried on the mucous membranes of the nose of a number of "healthy carriers" of the human population. In this way, the microorganism can be dispersed to other persons or the environment (Sonali Bhawsar, 2011). Additionally, some strains of this species can produce a powerful toxin that causes a vomiting response.

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**Biographical Sketch**

**Eugenia Bezirtzoglou -** With 25 years experience in the filed of microbial ecology of intestinal and food ecosystems, Professor Eugenia Bezirtzoglou has specific expertise in:
-Gastrointestinal microflora
-Anaerobic bacteria
-Food microbiology and hygiene
-Microbial ecology methods and techniques at cultural and molecular level
-Developing methods for sampling and culturing bacteria
-Designing experimental protocols to investigate the gastrointestinal ecosystem and factors influencing food microflora in health and disease.
In addition, she has been involved in many European (ECDC, EFSA) and National bodies (Ministries, Chemical State Laboratory) to offer her laboratory and teaching expertise on the above scientific fields.