AIR, SOIL & WATERBORNE MICROORGANISMS IN FOODS

1. Introduction

Microorganisms are present everywhere in our environment, in soil, air, food and water. Also called microbes, microorganisms are living organisms, generally observable only through a microscope. Bacteria live in almost any warm, moist environment, and there are thousands of different kinds. They are single-cell organisms that can reproduce very quickly and can spread through food, air or blood.

The transfer of contamination through the airborne route, soil and water body is one of the most significant areas of high-care food production. The food industry specially the manufacturing of the chilled meat products strive for lower levels of the air contamination, therefore lot of experimental and numerical studies considers the concentration of airborne particulate contaminants, such as different species of food spoilage microorganisms. The risk is higher when air is contaminated with eventually foodborne pathogen microorganisms and spores.

Airborne microbes are biological airborne contaminants (also known as bio aerosols) like bacteria, viruses or fungi as well as airborne toxins passed from one victim to the next through the air, without physical contact, causing irritation at the very least. This usually happens when an infected subject sneezes, coughs, or just plain breathes. It is hard to prevent such a method of transmission. Airborne microbes are a major cause of respiratory ailments. Mainly *Bacillus*, *Micrococcus*, and *Staphylococcus* are the microorganisms which are present in air.

Soil and water are common sources of important pathogenic and spoilage microorganisms, which is why it is important to thoroughly wash raw foods with good quality water. Air and dust are important sources of microorganisms during food processing and can influence food quality. *Bacillus subtilis* is mostly present in soil. Apart from this nitrogen-fixing bacteria, nitrifying bacteria, denitrifying bacteria and actinomycetes are also present in a huge quantity in soil.

Although most soil and water borne microbes will contaminate plants, very few types actually persist on them. Those that persist, such as lactic acid bacteria and some yeasts, must be able to adhere to the plant material and to utilize it for growth. Everyone has their own natural microorganisms that live on, in and around their own bodies. These bacteria are known as natural flora and our own bodies recognize that they are good for us. We, as humans, would not survive without such creatures.

2. Airborne microorganisms:

Bacteria have no active mechanisms for becoming airborne. They are dispersed on dust particles disturbed by physical agencies, in minute droplets of water generated by any process which leads to the formation of an aerosol. Many *actinomycetes*, especially those in the genus *Streptococcus* undergo this process. Air is mainly transport medium for microorganisms. They occur in small numbers in air when compared with soil or water.

The bacteria in the air consist both "good" (non pathogenic) and "bad" (pathogenic) bacteria, but most of them are good bacteria, and the levels of bad bacteria are low. The microflora of air can be studied under two headings; outdoor and indoor microflora.

Environmental factors that affect air microflora include atmospheric temperature (There is a progressive increase in the death rate with an increase in temperature from -18°C to 49°C), humidity (Low and high relative humidity cause the death of most microorganisms) and air current.

The majority of the airborne bacteria belonged to the genera *Bacillus*, *Micrococcus*, and *Staphylococcus*, but a total of 37 different genera were identified in the air. These results suggest that anthropogenic sources are major contributors to airborne bacteria: gram positive bacilli include:

- *Clostridium* species
- *Cornybacterium* species

Gram negative bacilli include:

- *Salmonella* species
- *Escherichia* species
- *Pseudomonas* species
- *Bacteroides* species

  Various *Micrococcus* like:

- *Micrococcus antarcticus; M. luteus; M. lylae; M. roseus; M. sp.*

2.1. Concentration of airborne microorganisms:

The air around us is filled with microbes. Bacteria, fungi, algae, protozoa, and viruses float in air currents. The numbers of microbes in the air range from 10 to 10,000 per cubic meter. They are found easily up to 3000 meters and as high as six miles into the air. According to a study, in Marseilles, concentrations of airborne viable microorganisms averaged 791 ± 598 bacteria m^{-3} (with a geometric mean of 536 ± 103 bacteria m^{-3}) and 92 ± 92 fungi m^{-3} (with a geometric mean of 63 ± 15 fungi m^{-3}). Airborne microflora, which increased a log-normal distribution in Marseilles, was shown to have a large variability. Airborne bacteria increase with temperature and wind velocity whereas airborne fungi increase with temperature and varied with wind direction in urban and natural areas. Partial identification of bacteria in Marseilles Island showed that geographical location Outdoor airborne microflora was investigated in urban and natural areas, the city of Marseilles had qualitative as well as quantitative influence on airborne microflora, this was illustrated by an increase of global airborne microorganisms, and more particularly Gram negative bacteria, in the urban area.

2.2. Morphology:

The word ***bacillus*** may be used to describe any rod-shaped bacterium, and such bacilli are found in many different taxonomic groups of bacteria. Bacilli are usually solitary, but can combine to form *diplobacilli*, *streptobacilli*, and palisades.

Micrococci have Gram-positive spherical cells ranging from about 0.5 to 3 micrometers in diameter and typically appear in tetrads. They are catalase positive, oxidase positive, indole negative and citrate negative. *Micrococcus* has a substantial cell wall, which may comprise as much as 50% of the cell mass. The genome of *Micrococcus* is rich in guanine and cytosine (GC), typically exhibiting 65 to 75% GC-content.

Micrococci can grow well in environments with little water or high salt concentrations. Most are mesophiles; some, like *Micrococcus antarcticus* (found in Antarctica) are psychrophiles. Though not a spore former, *Micrococcus* cells can survive for an extended period of time: unprotected cultures of soil *micrococci* have been revived after storage in a refrigerator for 10 years. Recent work by Greenblat *et al*. demonstrates that *Micrococcus luteus* has survived for at least 34,000 to 170,000 years on the basis of 16S rRNA analysis, and possibly much longer.

3. Soil borne microorganisms:

There is enough evidence in the literature to believe that microorganisms were the earliest of the living things that existed on this planet. Man depends on crop plants for his existence and crop plants in turn depend on soil and soil microorganisms for their nutrition. Scientists, from the beginning, studied the microorganisms from water, air, soil etc. and recognized the role of microorganisms in natural processes and realized the importance of soil microorganisms in growth and development of plants. Soil is considered to be the main source of scavenging the organic wastes through microbial action and is also a rich store house for industrial micro flora of great economic importance.

Soil microbiology emerged as a distinct branch of soil science during first half of the 19th century. Some of the notable contributions made by several scientists in field of soil microbiology are highlighted. J. B. Boussingault (1838) showed that leguminous plants can fix atmospheric nitrogen and increase nitrogen content in the soil. S. N. Winogradsky discovered the autotrophic mode of life among bacteria and established the microbiological transformation of nitrogen and sulphur. Isolated for the first time nitrifying bacteria and demonstrated role of these bacteria in nitrification (l890), further he demonstrated that free-living *Clostridium pasteuriamum* could fix atmospheric nitrogen (1893). He is known as “Father of soil microorganism”. B. Frank, i) discovered (1880) an *actinomycetes* “Frankia” (Actinorhizal symbiosis) inducing root nodules in non-legumes tress of genera *Alnus sp* and *Casurina* growing in temperate forests, ii) coined (1885) the term " Mycorrhiza" to denote association of certain fungal symbionts with plant roots (Mycorrhiza-A symbiotic association between a fungus and roots of higher plants. Renamed the genus Bacillus as Rhizobium (1889).

3.1. Bacteria in soil

Various microorganisms present in soil:

- **Nitrogen-fixing bacteria** form symbiotic associations with the roots of legumes like clover and lupine, and trees such as alder and locust. Visible nodules are created where bacteria infect a growing root hair. The plant supplies simple carbon compounds to the bacteria, and the bacteria convert nitrogen (N_2) from air into a form the plant host can use. When leaves or roots from the host plant decompose, soil nitrogen increases in the surrounding area. For example:- *Cyanobacteria, Rhizobia*.
- **Nitrifying bacteria** change ammonium (NH_4^+) to nitrite (NO_2^-) then to nitrate (NO_3^-), a preferred form of nitrogen for grasses and most row crops. Nitrate is leached more easily from the soil, so some farmers use nitrification inhibitors to reduce the activity of one type of nitrifying bacteria. Nitrifying bacteria are suppressed in forest soils, so that most of the nitrogen remains as ammonium. Nitrifying bacteria involves, *Nitrosomonas*, *Nitrobacter*, *Nitrosococcus.*
- **Denitrifying bacteria** convert nitrate to nitrogen (N_2) or nitrous oxide (N_2O) gas. Denitrifiers are anaerobic, meaning they are active where oxygen is absent, such as in saturated soils or inside soil aggregates.For example:- *Thiobacillus denitrificans, Micrococcus denitrificans, Paracoccus denitrificans.*
- ***Actinomycetes*** are a large group of bacteria that grow as hyphae like fungi . They are responsible for the characteristically "earthy" smell of freshly turned, healthy soil. Actinomycetes decompose a wide array of substrates, but are especially important in degrading recalcitrant (hard-to-decompose) compounds, such as chitin and cellulose, and are

active at high pH levels. Fungi are more important in degrading these compounds at low pH. A number of antibiotics are produced by *actinomycetes* such as *Streptomyces*.

3.2. Example of nitrogen fixing bacteria

Rhizobia are one of the groups of microorganisms living in soil. They are single celled bacteria, approximately one thousandth of a millimetre in length. *Rhizobia* belong to a family of bacteria called Rhizobiaceae. There are a number of groups of bacteria in this family.

Rhizobia belong to a specific group of bacteria that form a mutually beneficial association, or symbiosis, with legume plants. These bacteria take nitrogen from the air (which plants cannot use) and convert it into a form of nitrogen called ammonium (NH_4^+), which plants can use. The nitrogenase enzyme controls the process, called nitrogen fixation, and these bacteria are often called "nitrogen fixers".

Rhizobia are found in soils of many natural ecosystems. They may also be present in agricultural areas where they are associated with both crop legumes (like soybean) and pasture legumes (like clover). Usually, the *rhizobia* in agricultural areas have been introduced at sowing by applying an inoculum to the exterior of the seeds as liquid formations or pellets

The presence of numerous genera of spoilage bacteria, yeasts and molds, and an occasional pathogen on fresh produce has been recognized for many years. Several outbreaks of human gastroenteritis have been linked to the consumption of contaminated fresh vegetables and, to a lesser extent, fruits. Salads containing raw vegetables have been identified as vehicles of traveler's diarrhea, an illness sometimes experienced by visitors to developing countries. Enterotoxigenic *Escherichia coli* is the most common cause of this illness. Outbreaks of salmonellosis in humans have been attributed to consumption of contaminated tomatoes, mustard cress, bean sprouts, cantaloupe, and watermelon.

3.3. Fungi in soil:

Soil fungi are considered to be an important food source for earthworms. Fungal species (*Cladosporium cladosporioides, Rhizoctonia solani, Mucor* sp., *Trichoderma viride, Fusarium nivale, Phlebia radiata, Glaeophyllum trabeum, Coniophora puteana, Coriolus versicolor*), followed by fast-growing species such as *Mucor* sp. and *R. solan.*

3.4. Most natural antibiotics come from soil fungi and bacteria:

Many microorganisms have been playing a significant role long before they were discovered by man. Today, soil is considered to be the main source of scavenging the organic wastes through microbial action and is also a rich store house for industrial microflora of great economic importance. Soil bacteria and fungi live by digesting and recycling dead plant material such as leaves and seed. This material is typically carbon-rich and nitrogen-poor. Most common antibiotics are carbon-rich polymers made by enzymes that strongly resemble those that normally make saturated fats. The building blocks of these polymers are often exactly the same as those used to make saturated fats. Antibiotics are not that easy to find in microbes. Bacteria of the *Bacillus* genus occur mainly in soil and produce many widely studied antibiotic compounds. For example, *Bacillus subtilus* produces more than seventy-five known antibiotics consisting predominantly of small, cyclic peptides, but also including phospholipid, lipopeptide, and aminosugar antibiotics. It was hypothesized that antibiotic-producing bacteria would be of the genera *Bacillus* or *Streptomyces* and that the antibiotics would be peptides that inhibited the growth of Gram-positive bacteria.

4. **Waterborne microorganisms**:

There are many types of watery environments ranging from freshwater ponds, streams, puddles, lakes, rivers, and swamps to salty seas with three times the salt concentration of the ocean. Microbes live in overgrown slime, on pipes and in open oceans with few nutrients to support microbial life. Microbes thrive in streams with lots of oxygen to murky bogs that have no oxygen. In ponds there is a rich thriving ecosystem of microbial life including green and purple bacteria and algae, sulfate reducers, methane producers, and others. Many microbes live in the bottom of lakes and rivers in sediments. Many microbes cannot survive except in the presence of high concentrations of salt.

The largest watery place on earth is the ocean. Oceans cover 71% of the Earth's surface and are responsible for producing about half of the world's organisms, which includes the plants, animals, fungi, and microbes. Most life in the oceans lives at the sunlit ocean surface. Below 25 meters there is little light in the ocean, and life productivity decreases. As well as little light, deeper waters are cooler, which supports less life. Below 50 meters, the temperature is less than 10 degrees Celsius.

4.1. Microorganisms found in water:- water is also a habitat for various types of microorganisms, such as, bacteria, viruses, fungi and protozoans. *Campylobacter*, *Cholera*, *Cryptosporidium*, *Escherichia coli*, *Giardia*, *Hepatitis*, *Legionella*, *Protozoan parasite*, *Salmonella*, *Shigella* are commonly found in water.

4.1.1. Morphology:-

4.1.1.1. Campylobacter: These are gram negative, non-spore forming, microaerophillic bacteria. They are found in spiral and coccoid form and are distinguished from others due to their darting motility. Spiral form is found in young cultures and coccoid form is found in old cultures.eg: C. *jejuni*, *C.coli*, *C. laridis.*

4.1.1.2. Vibrio cholera: It is a gram- negative, , comma-shaped bacterium.*V. cholerae* is facultatively anaerobic and has a flagellum at one cell pole. *V. cholerae* was first isolated as the cause of cholera by Italian anatomist Filippo Pacini in 1854.

4.1.1.3. Cryptospordium*:* There are two species which are morphologically indistinguishable by light microscope examination namely *Cryptosporidium hominis*, *Cryptosporidium parvum.* Oocysts size ranges from 4-6 µm. It is round or oval in shape.

4.1.1.4. Giardia: It has two morphological stages: the trophozoite and the **cyst.**

- The trophozoite is pear shaped, with a broad anterior and much attenuated posterior. It is 10-12µm long and 5-7µm wide, bilaterally symmetrical, and has two nuclei. It is also relatively flattened, with a large sucking disk on the anterior ventral side, which serves as the parasite's method of attachment to the mucosa of the host. The trophozoite also has two median bodies and four pairs of flagella (anterior, caudal, posterior and ventral).
- The *G. lamblia* cyst is egg-shaped, and measures 8-14µm by 7-10µm. After encystation, each organelle duplicates, so each cyst contains four nuclei, four median bodies, eight pairs of flagella--although these organelles are not arraigned in any clear pattern. Upon excystation, each cyst produces two trophozoites.

4.1.1.5. Hepatitis: Among the smallest and structurally simplest of the RNA animal viruses. The virion is non enveloped and, with a diameter of 27-32 nm, it is composed entirely of viral protein and RNA. Electron microscopy analyses show particles with icosahedral symmetry although no structural details could be discerned. Morphologically, Hepatitis A virus particles are indistinguishable from other picornaviruses.

5. Fungi: These are a diverse group of organisms belonging to the kingdom *Eumycota*. This kingdom comprises five phyla, namely *Ascomycota*, *Basidiomycota*, *Zygomycota*, *Chytridiomycota*, and *Glomeromycota*. As a practical approach to classification, fungi have been divided into groups, such as the filamentous fungi, also called moulds, the yeasts, and the mushrooms. Some fungi are primarily adapted to aquatic environments, and will, therefore, naturally be found in water. These fungi are zoosporic, and many belong in phyla *Chytridiomycota*. Fungi belonging to the other phyla in *Eumycota* are primarily adapted to terrestrial environments.

5.1. Morphology:

Fungi are composed of filaments called hyphae; their cells are long and thread-like and connected end-to-end. Because of this diffuse association of their cells, the body of the organism is given the special name **mycelium**, a term which is applied to the whole body of any fungus. When reproductive hyphae are produced, they form a large organized structure called a sporocarp, or mushroom. This is produced solely for the release of spores, and is not the living, growing portion of the fungus.

In addition to being filamentous, fungal cells often have multiple nuclei. In the *chytrids* and *zygomycetes*, the cells are coenocytic, with no distinction between individual cells. Another feature of fungi is the presence of chitin in their cell walls. The chitin adds rigidity and structural support to the thin cells of the fungus, and makes fresh mushrooms crisp. Most members of the kingdom Fungi lack flagella; the structures are completely absent in all stages of their life cycle.

5.1.1. Ascomycota: Ascomycota is phylum of the kingdom fungi and, together with the Basidiomycota, form the sub kingdom Dikarya. Its members are commonly known as the sac fungi. They are the largest phylum of Fungi, with over 64,000 species. The defining feature of this fungal group is the "ascus", a microscopic in which nonmotile spores, called ascospores, are formed. The ascomycetes are a monophyletic group.

5.1.2. Zygomycota: The unique characteristic of the Zygomycota is the zygospore. Zygospores are formed within a zygosporangium after the fusion of specialized hyphae called gametangia during the sexual cycle. Single zygospore is formed per zygosporangium. Because of this one-to-one relationship, the terms are often used interchangeably. The mature zygospore is often thick-walled and undergoes an obligatory dormant period before germination. Most Zygomycota are thought to have a zygotic or haplontic life cycle. Thus, the only diploid phase takes place within the zygospore. Nuclei within the zygospore are believed to undergo meiosis during germination, but this has only been demonstrated genetically within the model eukaryote Phycomyces blakesleeanus.

Afterword

Microorganisms play a key role in maintaining life on earth, fixing gases and breaking down dead plant and animal matter into simpler substances that are used at the beginning of the food chain. Biotechnologists can also exploit the activities of microbes to benefit humans, such as in the production of medicines, enzymes and food. They are also used to breakdown sewage and other toxic wastes into safe matter. However, the above discussed pathogens and the types of disease caused by them makes clear that they are also agents of different diseases and these infectious agents not only spread through contaminated water and spoiled food but also through the omnipresent air and people with weak immune system becomes the first victims of these diseases. Control of these air, soil and water borne pathogens is important, and today many organisations like WHO is taking care of it. Many resistant bugs are found and controlling them is a new challenge for science. Strict adhesion to regulations and other standards set for basic hygiene maintenance and antibiotic use is a necessity

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