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SPECIFIC GUIDELINES ON " TAMING MICROORGANISMS "

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Introduction

Microorganisms are the smallest existing living things. They are measured in micros (1/1000 of a millimeter) and can only be seen through a microscope. They are everywhere, but they need adequate environmental conditions to grow and multiply. This group includes yeasts, bacteria, viruses and mould.
Some microorganisms cause diseases in people, animals or plants or alter foods, others have an essential role as oxygen producers, decomposing organic matter and mineralizing it or they help us to produce foods or take care of our health.

The existence of microorganisms was intuitively reported by the Greek and the Roman, but it was Antonie van Leeuwenhoek the first one to describe the yeast in beer in a scientific journal in 1680. Louis Pasteur published a paper on alcoholic fermentation in 1857, making yeast responsible for transforming the sugar in the must of grapefruit juice into alcohol.

Microorganisms y foods

There are certain foods, called fermented foods, where microorganisms have an essential role in their making. During the fermentation process the flavor, the period of preservation and the expiring date of the product are modified. Fermentation of foods was accidentally discovered and fermented foods have been consumed since the ancient times. Among fermented foods we can report wine, beer, bread, cheese, yogurt, kefir, cold meat, olives, and pickles.

There is archeological evidence on wine and bread making in the Neolithic (8.000 BC.), and beer is likely to have also been made by that time although the first archeological evidence was in the Sumerian times (3.500 A.C.).
Bread and beer were basic foods in ancient Egypt. The first written piece mentioning the use of vinegar was during the Roman Empire, when it was used to make different recipes. Cheese is likely to have appeared during the taming of animals in the Neolithic period, but the first graphic testimonies of cheese making dates back to 2,500BC in Mesopotamia, where a Sumerian frieze shows the production of cheese. Kefir was also consumed in the Sumerian times.
Although all these fermented foods have been produced since the ancient times, their making was spontaneous and there was no control over the process. Microorganisms are found on the surface of these foods, therefore they are responsible for the spontaneous fermentation processes that these foods undergo. In order to initiate fermentation of a new batch of foods, in an empiric way, a portion of the previously fermented food was stored and later mixed with the new food, making it possible to get new portions of fermented foods.
From the XIX century, when microorganisms were known to have a role in the different fermentation processes, the making processes started to become more reproducible. Currently we can select the right microorganisms depending on the characteristics that are required in the final product. We can produce the adequate microorganisms in large amounts, in large tanks called fermenters, preserve them through different techniques (freezing, refrigeration, lyophilize ation) and use them in the making processes of fermented foods, obtaining foods with homogenous characteristics among the different batches manufactured.

Bacteria in fermented foods.

Bacteria are unicellular microorganisms whose genetic material is not separated from the cell cytoplasm by a membrane. Bacteria are found in every habitat, including foods and the human body

Lactic acid bacteria

Lactic acid bacteria are part of a heterogeneous group of microorganisms, which are found in milk, meat products and vegetables. They produce lactic acid by a fermentation process of the sugars that are present in the foods where these bacteria live.

If the fermentation of sugars produces only lactic acid, the bacteria involved in the process are called "homofermentative" whereas if it produces ethanol or acetate and carbonic acid besides the lactic acid, the bacteria are called "heterofermentative". Lactic acid makes the

pH of food decrease, resulting in safer fermented foods because pathogenic microorganisms cannot grow or multiply in such conditions. In dairy and meat products acidification results in denaturalization of the proteins in food, therefore the food texture is modified. In dairy products acidification results in solidification, whereas in cold meats matter becomes more cohesive and resistant to chopping, making slicing easier.
Some lactic acid bacteria produce small-sized, protein-like compounds that inhibit growth or cause the death of the pathogenic bacteria or those bacteria that modify the properties of foods, making these fermented foods safer.
The activity of these bacteria on food sugars, fats and proteins makes these products easily digestible, originating compounds that are responsible for the taste and aroma that are specific to these products. Lactic acid bacteria have an essential role in red wine making. Although the main transformation in wine is the alcoholic fermentation caused by yeast, when the fermentation process is over and the yeast dies, some lactic acid bacteria (present in a very low concentration) can grow and transform the malic acid, which is present in most grape musts and is also responsible for the strong and rough taste, into lactic acid, reducing acidity and providing a much softer and pleasant taste.
During the process of pickle making (olives, eggplant, carrots, little onions, garlic, cabbage, capers, etc), vegetables are introduced in a saline solution, where there is a primary fermentation by lactic acid bacteria and yeast, which consumes most of the fermentable sugars and produces an acidification process that inhibits the growth of other bacteria. Later, there is a secondary fermentation process where the yeast that is resistant to acid consumes the rest of the sugars.

Acetic Acid Bacteria

Acetic acid bacteria are part of a heterogeneous group of long or rod-shaped microorganisms that are highly resistant to acid. These bacteria transform alcohol into acetic acid in the presence of oxygen. Besides acetic acid, they form small amounts of tartaric and citric acid. They can naturally be found in grapes and can undergo wine making conditions and still be active during wine storing. They need oxygen to grow and they produce great amounts of acetic acid. Traditionally, vinegar was naturally produced in wine barrels when wine soured spontaneously.

In 1864 Louis Pasteur explained, for the first time, the process produced by these bacteria. To avoid wine turning into vinegar, it is essential to control the growth of these bacteria (disinfection of the facilities, careful management of the must, correct wine production, and appropriate bottling). Currently, in vinegar industrial manufacture, it is used a type of acetic acid bacteria that can bear a high concentration of acid and a low concentration of nutrients to grow.

Micrococcaceae.

Micrococcaceae bacteria are round or coco-shaped and are present in all foods of an animal origin. They are used as ferments together with the lactic acid bacteria in fermented cold meet making (red and white cold sausage, salami, fuet). These bacteria grow at a low temperature and in the presence of salt. They contribute to stabilization of the color of cold meats, and their proteolytic and lipolytic activity contributes to their specific aroma and taste.

Yeast in fermented foods

Yeasts are unicellular sphere-shaped organisms whose genetic material is in a nucleus separated from the rest of the cell by a membrane. They were given that name because when they metabolize sugars they produce gas. Their size is twice the size of bacteria.
The most important yeast in foods is Saccharomyces cerevisiae, which is present in many environments but it can especially be found in foods of a vegetable origin and participates in beer, wine and bread making. These foods undergo an alcoholic fermentation process, where the yeast transforms the sugars present in flour, must and malt into ethanol and carbon dioxide. In bread, the carbon dioxide gets trapped into the dough, making it increase its size and conferring the bread its specific spongy texture. In wine and beer the carbon dioxide is released to the atmosphere, producing sparking bubbles in a similar way to a boiling liquid. Besides these main compounds, yeasts also produce components that contribute to the smell and taste of these foods.
Currently, although bread and wine making still uses natural yeast present in flour or must, beer undergoes a warming process during malting, resulting in the disappearance of most yeast, therefore it is essential that manufactured yeast should be added.
It is becoming quite common to use selected yeasts to ensure their presence and their effectiveness and the quality of the product, ensuring that the fermentation process is under control.

Yeasts die during bread baking but their components become part of this food. In wine and beer, when there are no more sugars, yeasts sediments on the bottom and, in most cases, we don't drink them because most of their cells are eliminated to obtain a transparent

drink. These cells will later be used for dietary supplements, like the brewer's yeast.
Another example of food in which yeast intervenes is kefir. In kefir different types of yeasts mix with lactic acid bacteria resulting in a spongy, whitish mass that ferments milk producing lactic acid, alcohol and carbonic acid.

Debaryomyces hansenii is yeast that intervenes in fermented cold meat making, improving their aroma and looks. It is especially adapted to dry, salty and slightly acidic environments. Unlike Saccharomyces, it does not produce a large amount of gas, because, if it did produced it, it would affect the product negatively (cold meats need to be compact and without air inside). Generally, yeast is added during the mixing of ingredients (minced meat and fat, salt and spices) so it can act inside, consuming the acids and softening the product taste, and metabolizing the fats which will produce components that will lend certain aroma and taste. Sometimes yeast is powdered on the surface of the tripe so that it gets a homogenous looks, avoiding cracks and other microorganisms growth.
Yeasts are also frequently found in all cheeses and brine because they are capable of growing in presence of salt, at a low temperature and they metabolize lactic and citric acid. Their proteolytic and lipolytic activity contributes to create aromatic components, to modify the product texture and to allow growth of other microorganisms.

Mould in fermented foods

Moulds are microorganisms whose genetic material is in a nucleus, separated from the rest of the cell by a membrane. During its multiplication the daughter cells do not separate from the stem cell, forming a structure called mycelia, which can be seen at sight.
They can only grow on the surface of the foods because they cannot grow in absence of oxygen. They are highly resistant to dry and acidic conditions. They form spores that allow growth under adequate conditions.
In dairy products there are two types of mould that contribute to the typical characteristics of these products. The blue mould (Penicillium roqueforti), which grows inside cheeses, like Roquefort, Cabrales or Gorgonzola. The white mould (Penicillium camemberti), which forms a cotton-like layer over the cheeses, like Camembert, Brie or goat cheese. Its proteolytic and lipolytic activity results in certain compounds that lend the taste and aroma of these products. In meat products, moulds develop on the surface, favoring the absence of cracks during the drying and dehydration process. Penicillium nalgiovensis is intentionally added on the surface of cured ham and fermented cold meats to avoid the growth of other moulds that generate toxins.

Viruses and foods

Viruses are microorganisms consisting of one molecule of nucleic acid and a protein capside. They inject their genetic material in the cells where the cell machinery is used to produce more viruses, which are freed to the medium when the infected cell breaks. There is a great variety of viruses and they infect nearly all living things: animals, plants, insects, and bacteria. If the microorganisms used to make fermented foods undergo a viral infection, their cells die and their capacity to transform the raw matter is altered, therefore the final product may be altered in its texture, taste and may not be safe any longer because of the likely growth of pathogenic microorganisms. The main way to avoid this from happening is 1) preventing contamination through adequate cleaning and disinfecting of the facilities, 2) using selected microorganisms that are resistant to viruses, and, since viruses always try to avoid such resistances, 3) keeping on replacing the selected microorganisms in order to stop further viral infections

Fighting pathogenic microorganisms through the use of viruses was suggested a bit after their discovery, but the discovery of antibiotics cut this research out. Nowadays, the increasing antibiotic-resistance of pathogenic bacteria is resulting in more scientific interest in this therapy.

There has been some research in order to develop new viruses with a lytic potential which is specific to the pathogenic bacteria so that the surfaces where products are manufactured can be disinfected, although its use has not been yet approved by the European Union.

Microorganisms and Health

A group of microorganisms permanently live in our organism, on our skin and mucosa, and they are called 'indigenous microbiota'. In this way we have a specific microbiota on the skin, another in the genital-urinary system, and another in the digestive system. The number of these microorganisms exceeds ten times the number of cells in our body and the relationship between the microbiota and our body is 'symbiotic' or beneficial for both.

These microorganisms ferment nutrients that we cannot use, producing short-chained fatty acids that favor the growth of epithelial cells in the intestine, vitamins like biotin, vitamin K and folic acid and they improve absorption of calcium, magnesium and iron. They also avoid pathogenic microorganisms settling and their presence contributes to boosting our defenses. Each person has a different and varied macrobiotic composition although everybody shares a series of common basic microorganisms. Factors such as a change in the diet, stress, antibiotic treatment may alter the microbial flora reducing the number of beneficial microorganisms and increasing the harmful bacteria, therefore improving the risk of suffering infections caused by pathogenic microorganisms.

The first studies about our microbiota were carried out at the end of the XIX century (In 1880 Theodor Escherich studied the microbiota in feces and its relation with digestion and in 1892 Albert Döderlein discovered the vaginal lactobacillus). From 2000, new methods (like next-generation sequencing methods) which allow 'reading' of DNA of all the microorganisms present in a sample without the need to grow them in the lab (many of them die in presence of oxygen), made it possible to study their diversity and functions more thoroughly.

Research on the intestine microbiota is one of the most active fields of current biology and scientists have just started to discover the complicated relationships that the microbiome (set of genes of the microbiota) has in the digestive system diseases (For example, Crohn's disease or irritable colon) or in other apparently unrelated diseases like obesity or diabetes.

Some doctors are searching new ways to modify the microbiome, especially in those extreme cases where bacterial infections, which are caused by resistant bacteria may result in death, through the "transplant of fecal matter.

The procedure is based on the introduction of the feces of a healthy person (with balanced microbiota), so that the microorganisms in the feces can colonize the sick person, replacing the harmful bacteria. In 94% of the cases the patient improves in a few hours. In the US there is the first bank that supplies the raw matter for these types of transplant.

At the beginning of the XX century, Iliá Metchnikoff was the first one in stating a theory that related ageing with the presence of toxic bacteria in the intestines. He attributed the longevity of the inhabitants in the Caucasus to the fact that they consume kefir and yogurt, making it possible to grow beneficial microorganisms in the intestine. This theory inspired Minoru Shirota who researched the relationship between well-being and bacteria, selecting the lactic acid bacteria that destroyed the dangerous bacteria that lived in the intestine, resulting in improved health. In 1935 he commercialized a fermented type of milk with this microorganism. It was called Yakukg, and it was the first marketed probiotic product.

Some years ago we had hardly ever heard of probiotics but now the word is familiar and we can find more and more products that contain probiotics and there is increasing scientific evidence that they are actually beneficial for consumers' health
In 2001 the World Health Organization (WHO) defined probiotics as those living microorganisms which, adequately administered, confer health benefits to the consumers. In 2002 an executive board in this organization provided the guidelines to assess probiotics in foods. Consumer's safety requires that health benefits should be supported by scientific evidence.

Probiotic microorganisms have a beneficial impact in many different ways. Some produce anti-microbial substances, others compete with pathogenic bacteria over nutrients or over the folds in the intestine walls, and others balance the patient’s immune system. In any case, in order to appreciate the beneficial effects, it is necessary to take probiotic microorganisms on a regular basis, because they go through the intestine tract without being part of host’s intestine microbiota**.** It has been proved that probiotic consumption (in fermented dairy products or in supplements) prevents acute diarrhea and improves diarrhea associated to the use of antibiotics. It also controls symptoms of inflammatory bowel diseases (Crohn's disease or irritable bowel syndrome) and it strengthens the immune system and helps in some types of allergies)
Most common probiotic microorganisms belong to a group of lactic acid bacteria called Lactobacillus, and to another group of bacteria called Bifidobacterium, but there are also probiotic yeasts like Saccharomyces boulardii (marketed as ‘Ultra-levura’), and a special strain that usually causes diarrhea (Escherichia choli), called Nissle 1917, marketed as 'Mutaflor' and it is used to prevent gastrointestinal diseases.

Effects of probiotics are specific to each microorganism, so in the last years, scientific research has been increasingly focusing on investigating the capacity of specific probiotic strains to protect the organisms and treat certain diseases.

Microorganisms as a cell factory

Industrial microbiology is a field that deals with the management and exploitation of microorganism for the production of substances and for the trading processes, and it involves considering the efficiency and profitability of the process. Although Louis Pasteur was the founder of scientific microbiology, Chaim Azriel Weizmann is

considered to have first performed industrial fermentation when he developed acetone production on a grate scale, benefiting from the metabolism of bacterium Clostridium acetobutylicum, and it was really important for the military industry during the Great War. Other industrial fermentation processes started developing as scientists discovered the nearly infinite microorganisms' metabolic skills.
A very important compound produced out of microorganisms is penicillin, which started the era of antibiotics. Before 1930, adults and children died, regardless their social class, from infectious diseases. Penicillin can cure diseases like tuberculosis, syphilis, pneumonia, rheumatoid fever, and made it possible to perform less risky surgical interventions. Alexander Fleming discovered it in 1928, when returning from his holiday he found that on a Staphylococcus aureus dish, there was a transparent area around a contaminating mould, which indicated cell destruction. This mould was later identified as Penicillium notatum, and the substance that produced the destruction of the bacteria was Penicillim G. Industrial production of penicillin emerged and flourished during Second World War.

Currently, the list of product and processes where microorganisms are used is nearly infinite and genetic engineering techniques (which started in the 70's) have allowed scientists to control and modify the microbial metabolism, improving production
A good example is penicillin: initially it was an expensive product and hard to isolate, with efficacy of 2 units per milliliter of culture, but at the end of the 80's it was easy to get 85.000 units per milliliter. Today penicillin is a pure chemical substance that can be sold and bought on a great scale in a competitive market.

Among the products that are produced using industrial microbiology processes, we have for the food industry: Amino acids (Glutamic, lysine, tryptophan, glycine, aspartic), vitamins (vitamin C, B2, B12) and proteins of microbial origin as food supplements. For the health industry: antibiotics, hormones (growth hormone and insulin), blood clotting factors, proteins and enzymes for diagnostic reagents and steroids and vaccines: industrial enzymes: hydrolytic (protease and cellulase for detergents), amylase, xylanase, as additives to animal feeds, glucose-isomerase for fructose production as sweetener; for the chemistry industry: alcohol (ethanol and methanol), acetone, polysaccharides (xanthene, dextran), fuels (ethanol, methane, hydrogen, propane, butanol) and raw matter to manufacture plastic; for the genetic engineering: restriction enzymes, polymerase and ligase

Besides generating all these products, which can be considered trading products, microorganisms take part in large scale process of great importance, such as environmental processes, especially urban and industry waste treatment; waste waters through biological treatment stages, decomposition of polluting substances generated by the industry, production of fertilizers and biological pesticides.

Potential topics for discussion

- How should we preserve genetic diversity in beneficial microorganisms?
- How are microorganisms preserved? Culture collection.
- How are main fermented dairy products manufactured?
- Are there harmful microorganisms in foods? How can we fight them?
- What pathogenic microorganisms can contaminate foods?
- How important are genetically-modified microorganisms in foods?
- How does intestinal microbiota impact in our health?
- How can we look after our intestinal microbiota?
- How can we get to know our microbiota better and its role in our organism?
- What do you know about the fecal transplant?
- What is the role of genetic engineering techniques in developing new biotechnological applications?
- How can genetically-modified organisms influence in nature?

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