Importance of microbial antagonisms about food attribution

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Some microorganisms can inhibit other microorganisms or reduce their growth in medium thanks to their metabolites, indirect (by changing pH, osmotic pressure and surface tension) or direct (by producing toxic component, antimicrobial component, bacteriocin, antibiotic etc.), this situation is called as antagonistic relation. These antagonistic interactions among microorganisms have been used often in biologic attribution of food. In recent years, depending on use of antimicrobial microorganisms and their metabolites in various food products, studies about stability and shelf life extension have gained a special importance. Nowadays, the most extensive research and commercial practices are based on lactic acid bacteria. In addition to lactic acid bacteria, it was reported that probiotics, yeast, fungus and bacteriophage have antagonistic characteristics. In this chapter, antagonistic relation among some microorganisms gradually increased importance about food attribution and often focused was explained.

Key words: Antagonism, food attribution, microorganisms

1. Introduction

In recent years, consumers prefer natural foods without chemical preservatives about food attribution. As a result of this situation, methods of natural protection begin to popular in food attribution and new preservation methods are focused on the use of microorganisms or of their metabolites; thus it was focused on antagonistic relation among microorganisms. In 1976 firstly by Antonie van Leeuwenhoek, antibiosis was mentioned as a biological interaction between two or more organisms that is detrimental to at least one of them or an antagonistic association between an organism and the metabolic substances produced by another. Also in 1877 Louis Pasteur and J.F. Joubert were reported that urine bacteria have inhibitor effect on Bacillus anthracis. In addition to these, it was found bacteriocins, antibiotics, lytic agents, bacteriolytic enzymes, bacteriophage and other metabolic products like hydrogen peroxide and diacetyl as other inhibitors produced by bacteria. These antagonistic interactions among microorganisms have been used often in biologic attribution of food. Lactic acid bacteria, yeast, fungus and bacteriophages have antagonistic characteristics and these are used on food attribution. Especially in recent years, depending on use of microorganisms and their antimicrobial metabolites in various food products, studies about stability and shelf life extension have gained a special importance. Nowadays, the most extensive research and commercial practices are based on lactic acid bacteria [1-10].

In this chapter, it was explained antagonistic relation among these important microorganisms gradually increased importance about food attribution.

2. Microbial Antagonisms

Some microorganisms can inhibit other microorganisms or reduce their growth in medium thanks to their metabolites, indirect (by changing pH, osmotic pressure and surface tension) or direct (by producing toxic component, antimicrobial component, bacteriocin, antibiotic etc.), this situation is called as antagonistic relation. For example, organic acids produced by microorganisms have antimicrobial effect on sensitive microorganisms to acids. Also some bacteria prevent growth of some other bacteria in media by producing metabolite called as bacteriocin. The most intensive studies and commercial practices about microbial antagonisms have focused on lactic acid bacteria. In addition to lactic acid bacteria, it was reported that probiotics, yeasts, fungus and bacteriophages have antagonistic characteristics [11-20].

2.1 Antagonistic characteristics of Lactic Acid Bacteria

Lactic acid bacteria are well known as beneficial bacteria and include probiotic bacteria that help maintain a good enteral environment and producers of biopreservatives in dairy products and fermented foods. It was determined in some researches that lactic acid bacteria have antimicrobial effects on some bacteria, pathogens, fungus and yeasts and also play an important role in detoxification of mycotoxins. Antagonistic effect of lactic acid bacteria is from their metabolites like lactic acids and other organic acids, H₂O₂, bacteriocin or antimicrobial components similar to bacteriocin, also diacetyl, ethanol and CO₂ [5,6, 16, 23, 24, 26, 27, 40, 44, 55, 56, 73, 84, 85].
2.1.1 Bacteriocins

Bacteriocins are bacterially produced antimicrobial peptides with narrow or broad host ranges. Antimicrobial effects of bacteriocins against sensitive microorganisms change depending on environmental factors like pH, temperature, composition and constitution of food [13, 14, 19].

Whereas bacteriocins are mostly synthesized by gram(+) bacteria, they are also produced by gram(-) bacteria. Bacteriocin produced by gram(-) is called as “micronin”. The size of protein, effect spectrum and mechanisms of microcins are different from bacteriocin produced by gram(+) bacteria. Whereas bacteriocin are known as an important biocontrol method in reduction of illness from food, some researchers were reported that bacteriocin can’t be used as first process step in pathogen inhibition, but it was noted that bacteriocin can be used with adding hurdle methods in preventing illness from food. Namely, it was determined that when bacteriocins are used with other protective components or attribution methods in food protection, they were more effective. It was mostly used bacteriocins synthesized by lactic acid bacteria in foods. Use of bacteriocin synthesized by lactic acid bacteria highly increased in food, because it has antimicrobial effect on most pathogen like Listeria monocytogenes, Staphylococcus aureus, Clostridium botulinum ve Salmonella spp. [25, 34, 35, 42, 47, 58, 69].

2.1.2 Organic Acids

Lactic acid bacteria especially some species like Lactobacillus, Lactococcus, Leuconostoc, Pediococcus and Streptococcus have been used as protective in fermented foods for long years. While homofermentative lactic acid bacteria produce lactic acid bacteria from hexose, heterofermentative lactic acid bacteria synthesized acetic acid beside lactic acid. Lactic acid bacteria have secured food safety and quality by reducing pH with these acids. Lactic acid are sour taste and odorless. It can mix easily with water and polymers, alcohol and ether; it is good solvent, weak acid. Lactobacillus species synthesized more lactic acid than Streptococcus species (51, 54).

Lactic acid bacteria have antimicrobial effect on pathogen bacteria like Escherichia coli, Staphylococcus aureus, Salmonella spp and Listeria monocytogenes in especially meat and dairy products, also spoilage bacteria, yeast and fungus in food product [68, 70, 71, 86, 80].

It was determined with several researches that both lactic acid and acetic acid synthesized by lactic acid bacteria have antimicrobial effects with wide spectrum on fungus, yeast and bacteria and also when there is propionic acid in media, antifungal effect of acetic acid increase [51, 54, 68, 70, 71].

2.1.3 Hydrogen Peroxide

Hydrogen peroxide (H₂O₂) is produced during aerobic growth of lactic acid bacteria and it is an oxidizing component. Hydrogen peroxide has antimicrobial effect on vegetative cells and spores of most microorganisms. Antimicrobial effect of H₂O₂ has occurred by changing chemical structure and biochemical characteristics of enzymes of target microorganisms. H₂O₂ inhibit most pathogen bacteria like Staphylococcus, Escherichia coli O157:H7, Pseudomonas and Clostridium, also spoilage. Most researches were shown that contents of H₂O₂ produced by lactic acid bacteria in optimum conditions is enough for inhibition of psychrophile bacteria [66, 83].

2.1.4 Laktoperoxidase

Laktoperoxidase is an enzyme that is found in secretion like milk, colostrum, salivary and lacrima. But, this enzyme is effective when there are H₂O₂ and thiocyanate (SCN⁻) in medium and thus it can show antimicrobial activity. In general it is known as laktoperoxidase system (LPS). Quantity of laktoperoxidase enzyme in cow milk is 3mg/100 ml or much more. Quantity in breast milk is less. On early days after birth, antimicrobial activity reaches to maximum level. While it show bactericidal effect against some bacteria, it show bacteriostatic effect against some. In general, this system is more effective on gr(-) bacteria than gr(+) bacteria. LPS show bactericidal effect on bacteria like C. jejuni, L. monocytogenes, Pseudomonas aeruginosa, Salmonella, S. aureus, ve Yersinia enterocolitica, also Coliform bacteria. It has been used as protective component in region that hygienic conditions is not good and during transport of raw milk from fabrics as non-cooler [16, 21, 67].

2.1.5 Diacetyl

Diacetyl is a product synthesized from pruvat as a result of metabolism of hexoses by lactic acid bacteria. Diacetyl has butter odor. It was found in most fermented product, especially dairy products and products from lactic acid fermentation. Homofermentative lactic acid bacteria are formed more diacetyl than heterofermentative lactic acid bacteria. Diacetyl show more inhibitor effect on gram(-) bacteria, fungus and yeasts than gram(+) bacteria. Also lactic acid bacteria are highly resistant to diacetyl [6, 16, 41, 50, 59, 63, 88].

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2.1.6 Ethanol
Ethanol is an antimicrobial metabolite produced by heterofermentative lactic acid bacteria. But in general production of ethanol is low during fermentation. Ethanol can’t be effective in food protection because it was inactivated by organic components in media [6, 16, 41, 50, 59, 63, 88].

2.1.7 CO₂
CO₂ is formed by most microorganisms via fermentation and has antimicrobial effect; also it is a fermentation product of heterofermentative lactic acid bacteria. Synthesis of CO₂ and antimicrobial effect occur in anaerobic conditions. In today, commonly it was utilize from antimicrobial effect of CO₂ in biocontrol of product packed in modified atmosphere. Gram(-) bacteria is more sensitive to CO₂ in modified atmosphere than gram(+) bacteria. Also lactic acid bacteria are more resistant than obligate aerobic microorganisms [6, 16, 41, 50, 59, 63, 88].

2.1.8 Antimicrobial Compounds with Low Molecular Mass
It was reported that some species of lactic acid bacteria synthesized compounds with low molecular mass. These compounds have antimicrobial activity and are active in low pH, thermostable, and can solve in acetone. Out of reuterin, reutericklin and 2-pyrolidone-5-carboxylic acid from these components, others are not identified and it hasn’t been found detail information about antimicrobial effect mechanisms yet [6, 16, 41, 50, 59, 63, 88].

2.1.9 Exopolysaccharides and Biofilms
A biofilm is an aggregate of microorganisms in which cells adhere to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance or exopolysaccharide (EPS). Exopolysaccharides (EPS) play an important role on bacteria resistance and form much level of biofilm. Many lactic acid bacteria are able to produce exopolysaccharides (EPSs). Most lactic acid bacteria that have probiotic characteristics and produce biofilm form protective effect against pathogenic bacteria. In recent years, it has been noted that biofilm produced lactic acid bacteria has antibacterial effect especially on some pathogen formed regard food safety in meat and dairy products. Researches were especially focused on antilisterial effect of biofilm formed lactic acid bacteria. Also, it was reported that biofilms produced by lactic acid bacteria are effective on fungus. But, it should be studied more about biofilms and focused on use of lactic acid bacteria biofilms as biocontrol agents with new studies in food industry [31, 46, 77].

2.2 Probiotics
Probiotics are live microorganisms thought to be beneficial to the host organism. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures; such as in yogurt, soy yogurt, or as dietary supplements. Consumption of probiotics has about benefits about healthy. Some these benefits include: enhancing bowel function, prevention of colon cancer, cholesterol lowering, lowering of blood pressure, improving immune function and reducing infections, reducing inflammation, improving mineral absorption, preventing growth of harmful bacteria, fighting off diseases like candida and exzema, and many more. Benefit about preventing growth of harmful bacteria show that probiotics have also antagonistic characteristics. Because of all these benefits of probiotics, it was reported that use of probiotic in food products will be helpful on food attribution and microbial quality of food [29, 30, 36, 37, 39, 62, 64].

2.3 Antagonistic Characteristics of Yeasts
Some yeasts having antagonistic effect against some fruit pathogens have been reported in 1980-1990. This antagonistic effect has shown by Debaromyces hansenii in citrus and some of the species belong to Cryptococcus in apples and other fruit has been reported [38, 61, 75, 76].

The main advantages of the antagonistic features of yeasts are list in; they don’t produce allergy-causing spores and mycotoxins and synthesize antimicrobial metabolites. In addition, nutrient requirements for growth of yeast are not very complex and even can be colonizing on dry surface for a long time. Use of yeast is economic compared with the other chemical antimicrobial because substrates used for growth are cheaper. In addition to the antagonistic effects of yeasts, yeasts are recommended for usage about food and animal feed because yeast cells have high amounts vitamins, minerals and essential amino acids [65, 78, 82, 87, 89].
2.3.1 Killer yeast

Some yeast synthesize toxin that has protein and glycoprotein structure and thus thanks to structure of toxin these yeasts inhibit other sensitive strains to toxin. This synthesized protein is named killer protein or killer toxin and so this yeast called as killer yeast. For the first time, killer yeast was found in *Saccharomyces cerevisiae* species by Bevan and Makower in 1963. This toxin is only effect on eukaryotic cell and has not an effect on human and prokaryotic cell. It is inactive at body temperature or degrades enzymatically (38, 87).

As known that some strains of yeasts have killer activity such as *Candida* (*C. valida, C. albicans*), *Cryptococcus* (*C. laurentii*), *Hanseniaspora* (*H. uvarum*), *Hansenula* (*H. anomala, H. mrakii, H. subpelliculosa*), *Kluyveromyces* (*K. marxianus, K. wickerhamii, K. phaffii*), *Pichia* (*P. kluveri, P. membranifaciens*), *Saccharomyces* (*S. cerevisiae, S. chevalieri, S. bayanus, S. capsensis, S. uvarum*). [76, 78, 82, 87, 89].

Killer yeast, in particular, becomes the dominant at spontaneous fermentation of wine. Because of these properties, when killer yeasts used as starter culture, controlled fermentation takes places. However, studies about selection as starter culture of these yeasts should make both in vivo and in vitro, because killer yeasts show different characteristics in different environments [82, 87, 89].

2.3.2 Some commercial yeasts used as bio-control products

Nowadays, commercial yeast is used as bio-control products especially in order to prevent to deterioration of fruits after harvest. The product known as “Aspire” has been used in the U.S. since 1996 and this product includes *Candida oleophila*. Aspire is used against deterioration during storage fruits such as citrus, apple and pear by spray or by immersion solution containing a certain concentration of yeast. “Yield plus” since 1997 in South Africa, have been used for bio-control of *Botrytis, Penicillium* and *Mucor* causing deteriorations on particularly varieties of apples and the other fruits. *Cryptococcus albidus* is antagonistic yeast in Yield plus. “Shemer” is another bio-control product based on *Metschnikowia fructicola* and has been described and approved in 2001 in Israel. It has broad use spectrum on fruits such as grapes, strawberries and sweet potatoes [11, 12, 38, 61].

It was utilized from antifungal characteristics of some *Debaryomyces* species during storage of fruit and dairy products. These yeasts have been used as commercial for inhibition of some fungus such as *Aspergillus* sp., *Byssoschlamys fulva*, *Byssoschlamys nivea*, *Cladosporium* sp., *Penicillium candidum* ve *Penicillium roqueforti* [75, 76, 78, 82, 87, 89].

2.4 Fungal Pigmens

Many studies have been done about the antioxidant and vitamins properties of the pigments synthesized by microorganism. Even today some of them are commercial production to be use in foods. The antimicrobial properties of microbial pigments have been noticed in recent years [43, 49, 53].

“*Monascus*” is the most widely known and used as food coloring pigment synthesized by some microorganisms. This pigment is from *Monascus* sp. and can pose ranging from yellow to orange colors. Especially, in the Far East, this pigment has been used for many years as a colorant in several foods such as wine, fish, cheese, beverages and even sausages. Antibacterial effect of a pigment synthesized by *M. purpureus* was determined against *S. aureus* with some studies. The antimicrobial effect of Monascus pigment varies according to type of target microorganism and pigment [43, 49].

On the other hand, for commercial purposes, carotenoid is obtained from *Dunaliella* spp., *Phaffia rhodozyma* and *Rhodotorula* species. In some studies it was reported that microbial carotenoids have different inhibition effects against food borne pathogens such as *Salmonella* Typhimurium S. Enteritidis, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* [43, 49, 53].

2.5 Bacteriophages

Firstly, the usage of bacteriophage as a bio-control factor proposed shortly after the discovery of bacteriophage independently by Twort (1915) and d’Herelle (1917) and suggested to be used in foods by Strauch *et. al.* (2007). Today, the studies of use of bacteriophages as a bio-control factor (phage therapy) revived because of increasing antibiotic resistances. Usually, these studies are concentrated about food safety, especially food pathogens such as *E. coli O157:H7*, *Campylobacter, Salmonella* and *Listeria* causing epidemics of disease. Recently, bacteriophages described “new” antimicrobial agents in food industry and studies carried out in order to expend the use of food [28, 32, 48].

Some researchers have two major concerns about the use of bacteriophages on food products. Consumption of these bacteriophages with food is whether harmful or not on human health and how to overcome phage resistance of bacteria against bacteriophages. There are some conditions to provide an effective bio-control by bacteriophages. When a phage and a host meet, there needs to be a match between structures on both, and this confers specificity on the interaction, i.e. any given phage will only infect a specific group of hosts. One of the remarkable factors in many food studies inactivation is only achieved by using a high phage to host ratio (the multiplicity of infection, or MOI) with food.
pathogens. In addition, inactivation has been reported to occur at temperatures beneath the growth minimum of the host. These facts suggest that the inactivation reported may be due to lyses from without, whereby large numbers of attached phages kill the cell via the ‘death of a thousand cuts’. An example which could be explained by this is the inactivation of Salmonella on melon which was reported to occur at 5°C and at an MOI of 200 [57, 60].

In general, studies in the literature are examined pathogens inactivation has been successfully by the use of bacteriophages. In a study published by Atterbury et al. (2003) was achieved rate of 95-99% success C. jejuni inactivation from chicken skin by bacteriophages. In another study has been reduced numerical of Listeria monocytogenes by phages in infected melon and soft cheeses. Similarly, in another study, Salmonella Enteritidis were followed after the application of phage therapy in raw and pasteurized milk and cheeses and were encountered in cheeses made form pasteurized milk after 89 days of storage. However, the presence of Salmonella Enteritidis is still determined in the same processed cheese made from raw milk. Similarly, in different study in order to inhibit of S. aureus in dairy products, researchers used lytic bacteriophage cocktail isolated from raw milk. The results have shown that bacteriophages can be use as a bio-preservative in manufacture of dairy products [52, 57, 60, 72, 74, 79].

In order to ensure food security, the first phage preparation to be used commercially contained a mixture of phages against Listeria monocytogenes. In the Federal Register of August 18, 2006, the US Food and Drug Administration (FDA) announced that it had approved the use of List-ShieldTM, the LMP-102 phage preparation made from six purified phages to be used on ready-to-eat meat and poultry products as an antimicrobial agent against L. monocytogenes. The approval of the phage preparation, developed from a company in USA, followed studies in which the effectiveness of the phage preparation against the pathogen on food was found and a spray application in meat and poultry processing plants was permitted. The approved phage cocktail has antimicrobial activity against 170 strains of L. monocytogenes. Former studies had shown that the phage cocktail is also effective on fresh fruits, like apple and melon, especially in combination with the bacteriocin nisin. Another phage, designated P100, was developed from a Netherlands company against L. monocytogenes and has also a potential to be widely used in the food industry. Listex P100 bacteriophage product received also approval from the FDA for use on cheese. The approval was granted under the FDA’s GRAS (Generally Recognized as Safe) procedure for use on cheese P100 was shown to be effective against a wide range of Listeria strains and a bio-informatic analysis of the total genomic sequence did not reveal any similarities of P100 genes or gene products to any genes or proteins or other factors known or believed to play a role in the pathogenicity or virulence of L. monocytogenes [28, 32, 48, 57, 60, 79].

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