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THE ROLE OF MICROBIAL CULTURE IN PRODUCTS INTENDED FOR HUMAN USE

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Abstract. *The paper presents a review of the role of microbial cultures in products intended for human use, with special reference to the use of bacteria and yeasts as working microorganisms. Microorganisms have a long history of application in food production, especially considering the products of alcoholic and lactic fermentation. With the development of technology, earlier spontaneous microbiological processes become controlled and guided to form acceptable physicochemical and organoleptic characteristics of food products. The application of microorganisms under certain environmental conditions can affect the consistency of the product, acidity, smell, taste, and color, but also the length of the shelf life, i.e. the sustainability of the product. Traditionally prepared cheeses, sour milk products, fermented sausages, beer, wines, and other products thanks to commercial microbial cultures can be produced in many batches and with established quality. Microbial biomass or extracted proteins can be dietary supplements or food ingredients. It should also be added the role of microorganisms in the production of raw materials such as enzymes, food flavors, and fragrances have an interchangeable role in the food industry and the production of general-use items. Probiotic microorganisms in food, dietary supplements, cosmetic and pharmaceutical products and their benefits for human health, prevention, and treatment of cardiovascular disease, obesity, type 2 diabetes, and cancer deserve special attention.*

Keywords: *food, microbial culture, probiotics, pharmaceutical products*

Introduction

Microorganisms are traditionally used in food processing and conservation. For thousands of years, microorganisms presented in used raw material have been involved in fermentations contributing to unique food properties. Variations in quality and organoleptic characteristics forced a careful selection of raw materials. Spontaneous fermentation is not possible to ensure uniform quality in different production batches mainly due to the variety of microorganisms naturally present in the raw material. In modern industrial production, starter cultures are used, i.e. precisely determined strains of microorganisms with detailed instructions for use. Starter cultures are selected depending on the probiotic properties, acid tolerance,

temperature range, salt tolerance [1] and other characteristics important for final product quality. Microbial culture in the processing of products for human use can be bacteria, yeasts and moulds. Moulds have applications in cheese production and the formation of authentic sensory characteristics. Selected bacteria and yeast participate mostly in lactic acid and alcohol fermentation. Tamang [2] reported that more than 5000 various fermented food products are consumed worldwide. Global Market Insights [3] reported that the fermented processed food market size (dairy products, bread and bakery products, vegetables, condiments, beverages) was valued at USD 105.8 billion in 2023 and is anticipated to register a Compound Annual Growth Rate (CAGR) of over 6.6% between 2024 and 2032.
Depending on the technological process and kind of product applied culture can be viable or destroyed in the final product. For example, in yoghurt starter cultures are presented in the final product, but in the production of pasteurised beer, the growth of the applied yeast's culture is stopped.
Some live cultures of microorganisms present in food have proven health benefits and are called probiotics. Thanks to the intensive marketing about probiotics' health and nutritional benefits, there is growing consumer demand for functional food products and dietary supplements containing probiotics. Based on the data of Grand View Research [4], the global probiotics market size was valued at USD 87.70 billion in 2023 and is expected to grow at a CAGR of 14.1% from 2024 to 2030.
The application of microbial cultures contributes to the design of products with modified sensory and physical-chemical characteristics. This paper is a review of some microbial cultures in the production of products for human use.

Probiotics

Human microbiota play an important role in maintaining a good state of health. Recently there has been an increasing number of different foods supplemented with probiotics and pharmaceutical products. Previously, most studies focused on probiotics' influence on improving and changing intestinal microbiota. Probiotics have become an adjunct to antibiotic therapy with various mechanisms of action (Figure 1).

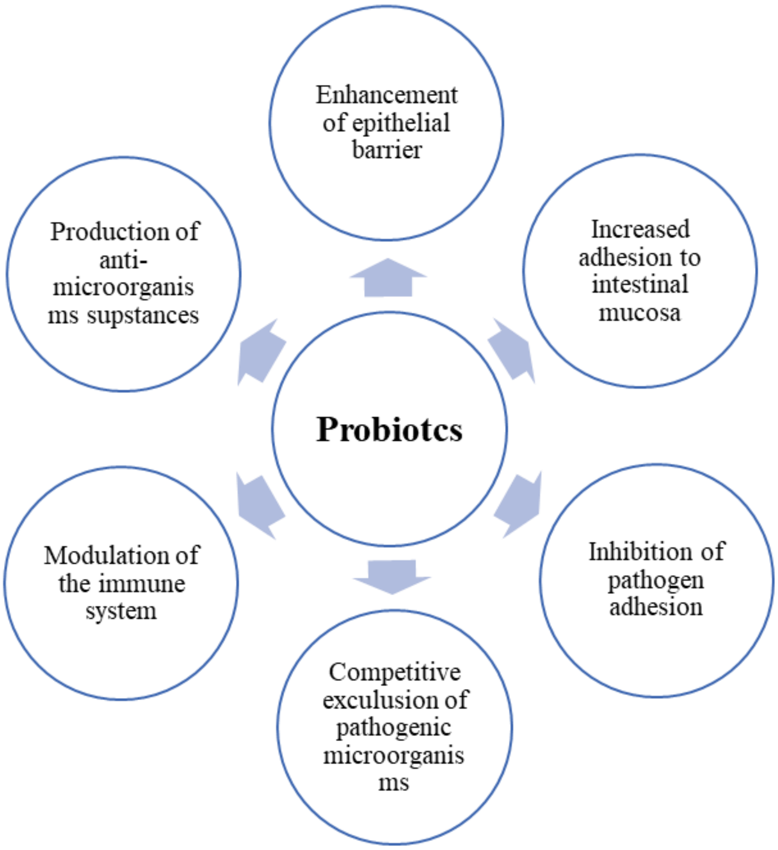

Figure 1. Mechanisms of action of probiotics [5]

So, probiotics are live microorganisms that confer a health benefit on the host. Depending on the food ingredients, the effectiveness of the realised probiotics varies. Recently, there has been growing attention on the positive health impact of probiotics on other organs. Cros-talk between the brain and present gut microbiota is an important spot in the pathophysiology of neurodegenerative disorders and regenerative processes [6]. For example, Duranti et al.[7] reported that *Bifidobacterium adolescentis* can stimulate the in vivo production of gamma-aminobutyric acid (GABA) highlighting their potential implication in gut-brain axis interaction.

The selection of a suitable food system to deliver probiotics is an important factor in the development of functional probiotic foods [8]. On the other side, many foods such as cereals, beverages and infant formulas are fortified with prebiotics to increase probiotic activity [9, 10, 11]. Many factors are affecting the efficiency of probiotics in the human body. For example, the physicochemical properties of food carriers have an important role in the survival of probiotics. Gastric acid is a huge barrier in the transit of probiotics. The buffering capacity and pH value have a significant role in the viability of probiotics.

Many bifidobacteria are commercialised as probiotics but the molecular mechanisms supporting their claimed probiotic action are not sufficiently explained [12]. Some of them have special abilities, for example, *Bifidobacterium intantis* utilises human milk oligosaccharides [13]. Yuniaty et al. [14] reported that *B. lactis* was more frequently detected in the stool of infants who received breast milk and probiotic-supplemented formula than in stool of infants who received breast milk and non-supplemented formula, both at 1 month and 2 months of age.

Table 1. Health benefits of some *Bifidobacterium*

Microorganism	Characteristics	Source
Bifidobacterium bifidum BF-1	Antibacterial features against *Helicobacter pylori*	[15]
Bifidobacterium breve	Antimicrobial activity against human pathogens Immuno-stimulating abilities Prevention/treatment of pediatric pathologies (gut diseases, including diarrhoea and infant colics, celiac disease, obesity, allergic and neurologic disorders)	[16]
	Supplementary strategy for the treatment of multiple sclerosis by increasing the antioxidant capacity and remyelination	[6]
Bifidobacterium infantis 35624	Relives many of the symptoms of irritable bowel syndrome (IBS)	[17]
Bifidobacterium longum	Relives IBS	[18]
	Potential to reduce the severity of or prevent certain diseases, including diseases linked with genetic, environmental, and lifestyle factors (non-communicable diseases, NCDs), in early life, across adulthood and into old age	[19]
Bifidobacterium longum BL-10	Excellent efficiency in preventing lipopolysaccharide (LPS)-induced acute liver injuri (ALI)	[20]
Bifidobacterium lactis HN019	Beneficial effects on lipid profile, beneficial effects on cytokines	[21]
Bifidobacterium adolescentis	Production of gamma aminobutyric acid (GABA), a principal inhibitory neurotransmitter with a key role in anxiety and depression disorders in mammals.	[22]
Bifidobacterium laterosporus BL1	Potential preventing strategy for obesity	[23]

Many strains of *Lactobacillus* are probiotics (Figure 2) and, after colonisation in the gastrointestinal tract, contribute to better health. Each species has unique biological activity and preventive/therapeutic applications. Also, *Lactobacillus* strains extend the shelf-life of foods suppressing the saprophyte and pathogen microbial growth.

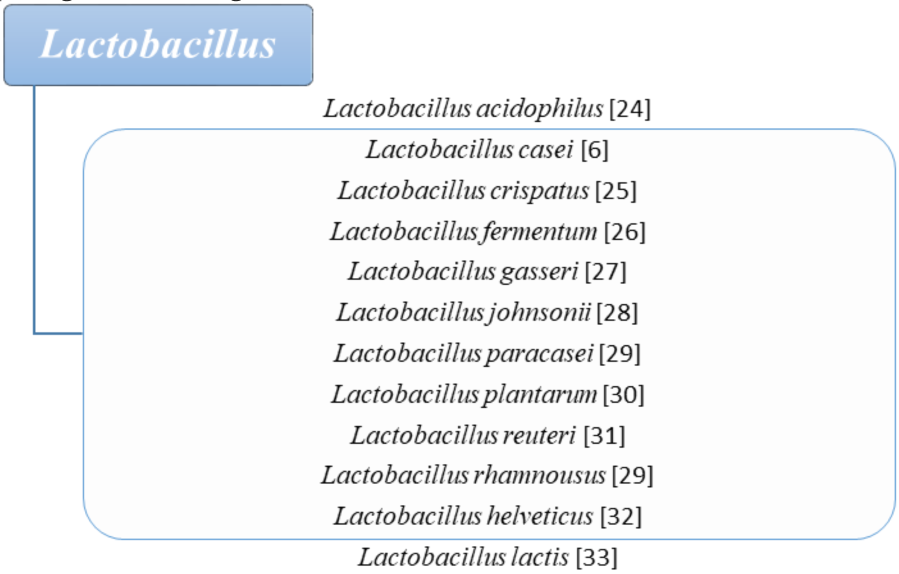

Figure 2. Probiotic species of *Lactobacillus*

Apart from *Lactobacillus* and *Bifidobacterium* dominating the probiotics market, other microorganisms are increasingly used as commercial probiotics. Although it is common to associate *Escherichia coli* with faecal contamination, the strain *E. coli* Nissle holds promise for the treatment of human disease. Pradhan et al, [34]. reported that Nissle was found that human intestinal tissues were not harmed by the Nissle bacteria introduced into the digestive, but conferred protection from pathogenic *E. coli* bacteria not by destroying it than protection likely occurred via the activation of human defences. A similar case is *Enterococcus faecium* SF68. Namely, Enterococci are present in high concentrations in human and animal faeces and are used as faecal indicators in aquatic environments. *E. faecium* SF68 has been shown to alleviate symptoms of intestinal inflammation in human clinical trials and animal feed supplementation studies [35]. Although probiotics are mostly bacteria, the yeast *Saccharomyces bulardii* has a significant application as a dietary supplement due to its wide spectrum of beneficial effects on human health (Figure 3).

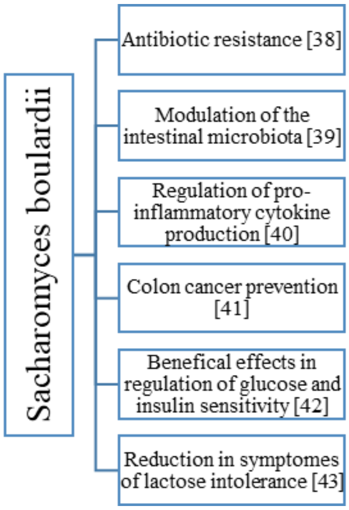

Figure 3. The benefits of *Sacharomyces boulardii* for human health

S. bulardii was discovered by French microbiologist Henri Bulard in 1920, who was in Indochina during a cholera outbreak. He noticed that people who did not develop cholera were drinking a special tea made from the outer skin of a tropical fruit and isolated the yeast he named “*Saccharomyces boulardii*” [36].

Recently, there is developed new probiotic-related views such as postbiotics and parabiotics, non-viable microorganisms or bacterial-free extracts with bioactivities additional to probiotics. Parabiotics are non-viable microbial cells and their fragments, also known as non-viable probiotics or inactivate probiotics. Postibiotics are non-viable bacterial products or metabolic byproducts by microorganisms in a matrix. Nataraj et al. [37] reported several advantages of parabiotics and postibiotics, including availability in their pure form and ease of production and storage.

Prebiotics are non-digestible oligosaccharides, fermented by probiotic bacteria to short-chain fatty acids. Prebiotics are present in several vegetables and fruits. Their addition during food production improves the sensory and textural characteristics of the food. Inulin and its hydrolysates, lactulose, maltooligosaccharides and fructooligosaccharides are prebiotics usually present in the human diet [53, 44].

Lactic acid and alcoholic fermentation

Humans used fermentation processes from ancient times before the biochemical processes were fully understood, and the role of microorganisms was discovered.

Lactic acid fermentation is a commonly used method of food conservation involving naturally present microorganisms or starter cultures. During fermentation, lactic acid bacteria (LAB) convert lactose into lactic acid, and consequently, pH decreases, proteins are degraded into peptides and amino acids, and lipids are transformed into free fatty acids. Usually, the concentration of lactic acid in fermented milk products is 0,6 to 1,2% [45] and there is not enough research on its influence on surviving other

microorganisms. Recently Đurđević-Milošević et al. [46] that 1% lactic acid water solution has bactericidal activity against *Pseudomonas aeruginosa, Escherichia coli* and *Salmonella* Enteritidis and also against *Salmonella* Typhimurium [47]. Other byproducts, such as carbon dioxide, various organic acids and alcohols can be produced as minor compounds. So, the results of lactic acid fermentation are physical and chemical changes forming the product with specific sensory characteristics and shelf life. Lactic acid fermentation is essential for forming fermented dairy, meat, and plant-origin foods.
Alcoholic fermentation is a complex process of reactions transforming sugars into ethanol, carbon dioxide and other metabolic byproducts. In this fermentation process, yeast is mostly used as culture. The benefits of alcoholic fermentation are in the production of bread, alcoholic beverages, biodiesel, pharmaceuticals and medical products [2].

Application of microbial culture and functional ingredients in industries

Dairy cultures play a key role in the transformation of milk into fermented milk products like yoghurt, kefir, cheese, and many other products, depending on the country or region of origin. *Streptococcus salivarius* subsp. *Thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* are a cultures for yoghurt production and are usually not part of the indigenous microbiota of mammals [48]. This is why some researchers do not recognise them as probiotics and are considered only as working microorganisms in dairy [49]. Selected bacteria, yeasts or moulds are used to produce dairy products to enhance taste, texture, and quality. One starter culture can show different growth rates and produced metabolites depending on the kind of milk [50]. Multicultured fermentation (*Streptococcus thermophilus*, 10% w/w *Lactobacillus bulgaricus*, 10% w/w *Lactobacillus acidophilus*, 10% w/w *Bifidobacterium* spp.) can be used for producing fermented products of cow's, goat's and soya milk. The addition of whey protein concentrates positively affects the syneresis, viscosity, and texture of the probiotic yoghurt produced [51]. Stijepić et al. [52] reported that the addition of honey and inulin to milk caused significantly lower syneresis ($p<0,05$) during storage time, while there was no significant influence on the viscosity and texture of the final product. The added functional ingredients during the fermentation, such as whey protein concentrates and honey, can improve the produced yoghurt's overall quality [53]. Inulin accelerated the fermentation process [44], while the honey addition shortened the fermentation time even faster [54, 55]. So, the production of fermented products with different functional can improve physicochemical and sensory characteristics [56, 57, 58, 59]. The increasing inulin content decreased the whey separation of probiotic products [60, 61] and contributed to better human health and product properties also (Figure 4).

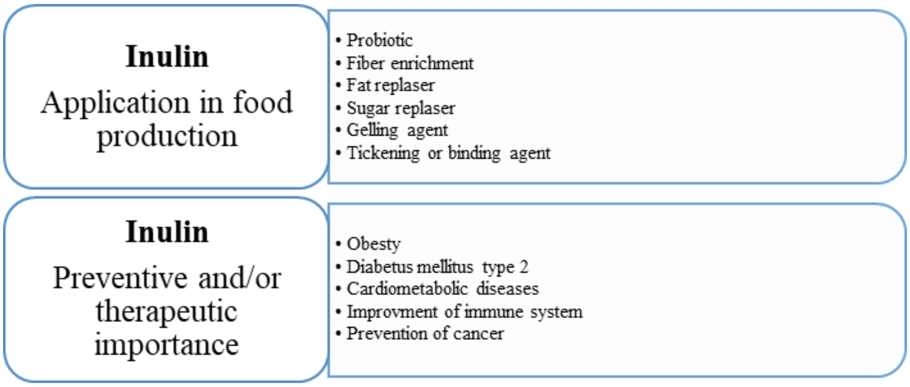

Figure 4. Application of inulin in food production and health benefits [54]

In the production of cheeses predominantly LAB is used, yeasts and moulds also can be involved. The interesting process production of blue cheese Roquefort implies the use of mesophil LAB (*Lactococcus lactis*, *Lactococcus cremoris*, and *Leuconostoc* spp.). Traditionally produced cheese became naturally contaminated with the fungus *Penicillium roqueforti*, but in industrial production adding commercial spores is common practice.

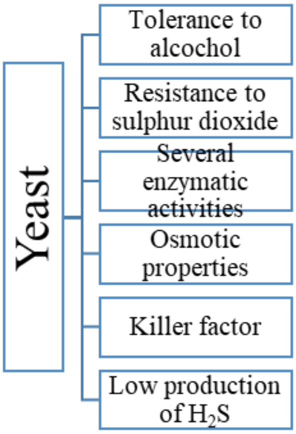

Figure 5. Selection criteria for yeast in wine production [62]

Yeasts, mainly *Saccharomyces cerevieisae*, have a main role during the alcoholic fermentation of grapes and winemaking, Specific criteria have been used to evaluate proper *S. cerevisiae* starter culture with oenological properties [62] (Figure 5).

Based on the fermentation process, beer is divided into top-fermentation bear and bottom-fermentation bear, with various characteristics of colour, taste, alcohol content and even shelf life. *Sccharomyces cerevisiae* is considered a top-fermenting yeast since it remains in the fermentation vessel. The temperature for its optimal growth and quality bear is 18-27 ^{o}C. Unlike *S. cerevisiae*, *S. pastorianus* is bottom

fermenting yeast because flocculates naturally and settles on the bottom of the container throughout the fermentation. *S. pastorianus* is an allopolyploid hybrid from *S. cerevisiae* and *S. eubayanus* adapted to low temperatures of fermentation (5-15 ºC). The main characteristics of bears are formed during fermentation, but some physicochemical changes are present even after pasteurisation during storage time [63, 64, 65]. *S. cerevisiae* as baking yeast is row material in bakery.
In addition, fermentation and microorganisms have an important role in the production of fermented vegetables. Microorganisms are also used in the pharmaceutical industry in the production of antibiotics, acids, enzymes, antibiotics and other active substances for human use.

Conclusion

For centuries, microorganisms have played an important role in the preservation and formation of food characteristics, as well as the production of metabolites that are important for further technological processes and/or human health. With the development of biotechnology and the pharmaceutical industry, strains of microorganisms, that can be used to control the production process and form products with uniform characteristics, have been isolated and recognised for further application. Microorganisms that have a dominant application in the food industry are lactic acid bacteria and the yeast *S. cerevisiae*. Some microorganisms and their metabolites can have positive effects on the human microbiome and thereby contribute to better health. Such strains are used for the production of dietary supplements and in the pharmaceutical industry to obtain certain metabolites for human use.

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ULOGA MIKROBNE KULTURE U PROIZVODIMA NAMJENJENIM ZA LJUDSKU UPOTREBU

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Sažetak. *U radu je dat pregled uloge mikrobinih kultura u proizvodima namjenjenim za ljudsku upotrebu, sa posebnim osvrtom na upotrebu bakterija i kvasaca kao radnih mikroorganizama. Mikroorganizmi imaju dugu istoriju primjene u proizvodnji hrane, posebno u proizvodima alkoholne i mliječne fermentacije. Razvojem tehnologije, raniji spontani mikrobiološki procesi postaju kontrolisani i vođeni da formiraju prihvatljive fizičko-hemijske i organoleptičke karakteristike prehrambenih proizvoda. Primjena mikroorganizama u određenim uslovima sredine može uticati na konzistenciju proizvoda, kiselost, miris, ukus i boju, ali i na dužinu trajanja, odnosno na održivost proizvoda. Tradicionalno pripremljeni sirevi, proizvodi od kiselog mlijeka, fermentisane kobasice, pivo, vino i drugi proizvodi zahvaljujući komercijalnim mikroobiološkim kulturama mogu se proizvoditi u više serija i sa utvrđenim kvalitetom. Mikrobna biomasa ili ekstrahovani proteini mogu biti dijetetski suplementi ili sastojci hrane. Takođe treba dodati i ulogu mikroorganizama u proizvodnji sirovina kao što su enzimi, arome hrane i mirisi koji imaju zamjenjivu ulogu u prehrambenoj industriji i proizvodnji predmeta opšte upotebe. Posebnu pažnju zaslužuju probiotički mikroorganizmi u hrani, dijetetskim suplementima, kozmetičkim i farmaceutskim proizvodima i njihove koristi za zdravlje ljudi, prevenciju i liječenje kardiovaskularnih bolesti, gojaznosti, dijabetesa tipa 2 i raka.*

Ključne riječi*: hrana, mikrobne kulture, probiotici, farmaceutski proizvodi*