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# THE APPLICATION OF PROBIOTIC BACTERIA IN THE FERMENTED VEGETABLE, CEREAL AND MEAT PRODUCTS*

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Abstract

Probiotic microorganisms are known to have the beneficial effects on people's health. Literature data show that the best source of probiotic bacteria are dairy fermented products. Not only dairy fermented foods contain probiotic cells. There are also non-dairy products from fermented vegetables, cereals, soy and meat where a growth of probiotic bacteria takes place. The interest in the application of probiotic bacteria in the food production comes from their positive influence on human organisms. They are applied in the treatment of gastrointestinal infections, virus and bacteria infections. Probiotic bacteria improve the lactose metabolism and decrease cholesterol. They possess anti-mutagenic and anti-carcinogenic properties. There is a need of widening a variety of non-dairy products in which it will be possible to apply probiotic bacteria.

The purpose of this paper was to discuss the possibilities of application of probiotic bacteria in new and traditional non-dairy products on base of literature data.

## ZASTOSOWANIE BAKTERII PROBIOTYCZNYCH W FERMENTOWANYCH PRODUKTACH WARZYWNYCH, ZBOŻOWYCH I MIĘSNYCH

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Słowa kluczowe: produkty niemleczarskie, bakterie probiotyczne, funkcjonalne produkty probiotyczne.

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Abstrakt

Mikroorganizmy probiotyczne mają korzystny wpływ na zdrowie człowieka. Dane z piśmiennictwa wskazują, że najlepszym ich źródłem są fermentowane produkty mleczne. Nie tylko one zawierają bakterie probiotyczne. Istnieją również fermentowane produkty otrzymane z warzyw, zbóż, soi oraz mięsa, w których występuje wzrost bakterii probiotycznych. Zainteresowanie zastosowaniem tego rodzaju bakterii w produkcji żywności wynika z ich prozdrowotnego wpływu na organizm ludzki. Stosuje się je w leczeniu infekcji układu pokarmowego, zwalczaniu infekcji wirusowych i bakteryjnych. Bakterie probiotyczne wspomagają metabolizm laktozy oraz obniżają poziom cholesterolu. Mają właściwości antymutagenne i antykancerogenne. Istnieje potrzeba powiększenia oferty produktów niemleczarskich, w których możliwe będzie zastosowanie bakterii probiotycznych.

Celem pracy było omówienie możliwości zastosowania probiotyków do wyrobu nowych i tradycyjnych produktów uzyskanych z warzyw, zbóż, soi i mięsa na podstawie danych literaturowych.

## Introduction

Probiotics are commonly known to have the beneficial influence on the host organism (BROWN and VALIERE 2004). They positively affect the gastrointestinal infections, possess antimicrobial activity, help with lactose metabolism, decrease serum cholesterol, stimulate immune system, possess antimutagenic properties, anti-carcinogenic properties, anti-diarrheal properties, alleviate the inflammatory bowel disease symptoms, suppress the infection caused by *Helicobacter pylori* (AGERHOLM-LARSEN et al. 2000, GOTCHEVA et al. 2002, NOMOTO 2005, IMASSE et al. 2007, SHAH 2007).

They are usually added to yogurt and other fermented dairy products (LOURENS-HATTINGH and VILJOEN 2001, PENNA et al. 2007). However, in the recent years, there has been the increasingly observed demand for so called non-dairy-based probiotic products. Such microorganisms are often added to drinks or sold in the form of tablets, capsules and freezedried formula (SCHREZENMEIR and DE VRESE 2001). Probiotic microorganisms inhabit human and animal intestines. It is known that fermentation is used to preserve products of plant and animal origin, improve their quality, change their flavor. Traditional fermented foods contain a great number of probiotic microorganisms (DE VALDEZ et al. 1990, ASHENAFI and BUSSE 1991, BOTES et al. 2007, LEE and LEE 2006, LEI and JACOBSEN 2004, PSANI and KOTZEKIDOU 2006, TODOROV et al. 2008). The consumption of these products is strongly recommended due to their ability to help with lactose intolerance and cholesterol decrease.

## Probiotic bacteria

Probiotic bacteria are commonly consumed with fermented products, and dairy food is a main source of probiotics (HELLER 2001). These microorganisms act beneficially when they are delivered as viable cells. They need to survive in non-fermented food matrices as well as non-dairy products (Sheehan et al. 2007). Probiotics belong not only to the genera *Bifidobacterium* and *Lactobacillus,* but also to the genera *Lactococcus, Enterococcus, Saccharomyces* and *Propionibacterium* (Blandino et al. 2003, Vinderola and Reinheimer 2003). They inhabit human and animal gastrointestinal tract. The intestines of newborns are exclusively colonized with *Bifidobacterium* within a few days after birth. The ability of probiotic bacteria to colonize the intestines and influence unwanted intestinal bacteria is dependent on many factors. The optimum pH for the growth of *Bifidobacteria* is in a range of 6.0–7.0 which means that no growth takes place below of 4.5 and above 8.5. The optimum growth temperatures are 37–41°C, the minimum are 25–28°C, and the maximum are 43–45°C. Among *Bifidobacterium* there are the most commonly mat strains which are *B. adolescentis, B. longum, B. infantis*, and *B. breve*.

Probiotic bacteria are often belong to the LAB genera and are classified on the base of their morphology and an ability to glucose fermentation. They are commonly applied in the food industry due to their ability to convert fermentable sugars into lactic acid, ethanol and some other metabolites, which are responsible for lowering the pH value and preventing the potential growth of pathogenic microorganisms in food products as well as in the human intestines. These bacteria are classified as homofermentative producing lactic acid as main metabolite, and heterofermentative producing ethanol and carbon dioxide. It is known that some LAB strains may be used as probiotics because they tolerate the conditions present in the host gastrointestinal track and they are able to prevent the growth of undesirable pathogenic bacteria (Chiu et al. 2007). Among LAB the most significant are *Leuconostoc, Lactobacillus, Streptocccus* and *Pediococcus.* In the genera *Lactobacillus*, *Lactobacillus acidophilus* is known to be in a majority in the intestinal tract of healthy people. It is very often applied in probiotic products (Arihara et al. 1998). It is able to grow in temperature 45°C and its optimum is in a range of 35 and 40°C. *L. acidophilus* multiplies in slightly acidic conditions in pH of 6.4–4.5, and survives in pH of 4.0–3.6. It stays alive in 0.3% to 1.9% titrable acidity, while its optimum pH is 5.5–6.0 (Curry and Crow 2003).

Probiotics are used for the production of functional foods which means that they have to be viable and available at a high concentration, usually at least $10^8$–$10^9$ per gram of product. They also need to tolerate the presence of human gastric juice in the stomach and be able to colonize the small intestine and the colon. They influence beneficially the intestinal immune system, eliminate enteric pathogens, deliver antimutagens and antioxidants (Park et al. 2007). It should be taken into consideration that each strain possesses completely different beneficial features (Shah 2007). Probiotic bacteria should tolerate the severe conditions in the gastrointestinal track. They must be resistant to acid, bile, enzymes, low levels of oxygen. Such the resistance to bile acids ensures that probiotic bacteria reach the intestinal tract as alive cells (TARANTO et al. 2006). Probiotic bacteria must be able to adhere to mucosal surfaces of the intestines.

The antimicrobial features of LAB and *Bifidobacterium* are related to the ability to compete for nutrients and the ability to produce the inhibitory substances including organic acids, hydrogen peroxide and bacteriocins. The capability of decreasing pH value as a result of the production of organic acids contributes to a bacteriocidal and bacteriostatic effect (SHAH 2007). Probiotic bacteria due the acid production eliminate the pathogens from the host intestines (OUWEHAND and SALMINEN 1998). The presence of lactic acid in combination with bile salts causes the growth inhibition of Gram-negative pathogenic bacteria (BEGLEY et al. 2005). The biological activity of probiotics added to fermented foods is dependent on some factors. They include the physiologic state of the probiotics given by the logarithmic or the stationary growth phase; the concentration at the time of consumption related to the fact that some commercial products do not contain a proper number of viable probiotic bacteria during their shelf-life (DAVE and SHAH 1997, SCHILLINGER 1999); the chemical composition of the product including its pH, water activity, the carbon, nitrogen, mineral and oxygen content, the possible interactions of the probiotics with the starter cultures (TRACHOO et al. 2006).

## Vegetable products

Dairy products are regarded to be the current industrial probiotic foods (HEENAN et al. 2004). Due to the technological advances it is possible to change some structural features of fruits and vegetables by the introduction of some component modifications in a controlled way (BETORET et al. 2003). Such modification makes it possible to be ideal substrates for the probiotic bacteria because of a high content of minerals, vitamins, dietary fibers, and antioxidants (YOON et al. 2004). There is a great need to produce fruit functional drinks containing probiotics. They enjoy a huge popularity due to their taste suitable for all age groups and because they are considered to be healthy and refreshing (TUORILA and CARDELLO 2002, SHEEHAN et al. 2007). When drinks are added with *Lactobacillus plantarum*, the unsuitable aromas (perfumery, dairy) and flavors (sour, savory) are felt (LUCKOW and DELAHUNTY 2004). Unfortunately, it has been discovered that customers more eagerly consume juices having conventional favour in comparison to the ones containing probiotics). Their interest grows when they are provided with the information on the beneficial value of such juices.

It is known that LAB are a group of bacteria having special requirements and they need essential amino acids and vitamins for their growth (SALMINEN and VON WRIGH 1993). On the other hand, it has been discovered that there are some probiotic bacteria which can grow in fruit products. The ability of cells to grow is dependent on the strains, feature of the substrate, the oxygen content as well as the final acidity of product. The addition of *Lactobacillus* and *Bifidobacterium* into orange, pineapple and cranberry juices caused huge differences in the characteristics of juices. All of the strains added to the juices had an ability to survive for a longer period of time in orange and pineapple juices in comparison to cranberry. *Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus paracasei* were able to survive at levels above 7.0 log cfu/ml in orange juice and above 6.0 log cfu/ml in pineapple juice for at least 12 weeks. However, the thermal pasteurization at 76°C for 30 s and 90°C for 1 min in combination with a high-pressure treatment of 400 MPa for 5 min, *Lb. casei, Lb. rhamnosus* and *Lb. paracasei* were not able to withstand a level above 6.0 log cfu/ml (SHEEHAN et al. 2007).

LAB cause the fermentation in vegetables and their composition depends on the quality of the raw material, temperature, as well and harvesting conditions (ROBERTS and KIDD 2005, YOON et al. 2006). The fermentation in vegetables is mainly carried out by *Lb. plantarum, Leuc. mesenteroides* (CLEVELAND et al. 2001, YAN et al. 2008) and *Lb. paracasei/casei, Lb. delbrueckii* and *Lb. brevis* (CZYZOWSKA et al. 2006). Different strains of probiotic bacteria have been isolated from vegetables. Fermented olives have contained *Lb. casei* in its majority (RANDAZZO et al. 2004). Species belonging to genus *Leuconostoc* encourage *Lactobacilli* and *Bifidobacteria* to grow in the intestines due to its ability to produce dextransucrase (EOM et al. 2007, SANZ et al. 2006). It has been proved that tomato juice constitutes a good substrate for *Lb. acidophilus, Lb. plantarum, Lb. casei* and *Lb. delbrueckii* to carry out the lactic acid fermentation despite its low pH value (YOON et al. 2004). After fermentation pH of tomato juice decreases from an initial pH value of 4.1 the pH 3.5 after 72 h. Bacteria multiply very rapidly and in 30°C after 48 h are able to achieve a number of $10^8$ cfu/ml from in comparison to an initial number of $10^5$ cfu/ml.

It has been indicated that *Lb. acidophilus, Lb. plantarum, Lb. casei* and *Lb. delbrueckii* were viable and in a proper number in fermented tomato juice having high acidity. Such juice may be called a probiotic beverage because it contains viable LAB after 4 weeks of keeping it at 4°C, and *Lb. acidophilus* and *Lb. delbrueckii* stay in dominance. YOON et al. (2006) applied the same LAB in a form of *Lb. plantarum, Lb. casei, Lb. delbrueckii* to check if cabbage may constitute a good substrate for their life and thus if cabbage may be used for production of probiotic cabbage juice. It was observed that *Lb. plantarum, Lb. casei*, and *Lb. delbrueckii* were able to multiply fast in sterilized cabbage juice. Their number increased up to $10^8$ cfu/ml after 48 h of fermentation at 30°C. For better fermentation in vegetable juices and better multiplication of probiotic bacteria, they are recommended to be enriched with brewer's yeast autolysate (RAKIN et al. 2007). It was found that red beets are a good substrate for probiotic bacteria. KYUNG et al. (2005) investigated if *Lb. acidophilus* and *Lb. plantarum* could multiply in the beet juice. The experiment proved that both strains decreased the pH value of beet juice from 6.3 to 4.5 after 48 h of fermentation. They produce significantly more lactic acid in comparison to *Lb. casei* and *Lb. delbrueckii*.

To sum up, the growth of probiotic bacteria is dependent on a vegetable used, on a strain used, on the final acidity of the product as well as the concentration of lactic and acetic acid in the vegetables (DAVE and SHAH 1997, YOON et al. 2004). Probiotic bacteria due to their beneficial features for human organisms should be commonly added to vegetable products.

## Cereal and soya products

Cereals are commonly known to be an extremely rich source of protein, carbohydrates, vitamins, minerals and fiber in a diet of people (CHAVAN and KADAM 1989). What is more, due to their high content of non-digestible carbohydrates they play a role of prebiotics encouraging the growth of *Lactobacilli* and *Bifidobacteria* in the intestines (ANDERSSON et al. 2001). Their prebiotic features are supported by the fact that they contain water--soluble fiber, oligosaccharides and resistant starch (SHAH 2001, ANDERSSON et al. 2001). Apart from these components they also contain phytoestrogens, phenolic compounds, antioxidants, phytic acid and sterols (KATINA et al. 2007). It is known that cereals possess a lower nutritional value in relation to milk and its products. They contain a lower protein content, they lack some essential amino acids, they show low starch availability. The fermentation process causes a decrease in amount of carbohydrates and oligosaccharides, causes some protein degradation leading to releasing some amino acids like lysine and helps with the synthesis of vitamins from B group. The fermentation with its relatively high acidity is responsible for releasing mineral compounds like manganese, iron, zinc as well as calcium which help LAB grow (BLANDINO et al. 2003).

As cereal grains are a rich source of fermentable carbohydrates, amino acids, B vitamins, nucleic acids and minerals, they may constitute an ideal substrate for bacteria belonging to *Lactobacillus* genus. Moreover, fermented cereal products may be called functional products containing viable cells of probiotic bacteria. Some strains such as *Lb. plantarum, Candida rugosa* and *Candida lambica* were identified from microflora living in a traditional Bulgarian cereal-based fermented beverage giving it a status of a probiotic product. These strains were found not to be sensitive to 2% bile concentration and such feature makes them possible to stay viable in the gastrointestinal system (Gotcheva et al. 2002). The cereal products constitute a good medium for the growth of probiotic bacteria thus making them functional products. They possess a suitable chemical composition as their malt, wheat and barley extracts are ideal for the growth of *Lb. plantarum, Lb. fermentum, Lb. acidophilus* and *Lb. reuteri*. It should be noted that cereal grains are a good environment not only for the growth of LAB but also for yeast. The relationships between these microorganisms need to be discussed as they have a huge influence on themselves causing the stimulation or inhibition of some strains. There is a competition among microorganisms about nutritional substances and they release some metabolites which may inhibit or encourage the growth of other microorganisms. It is known that yeast may be producers of vitamins which help LAB grow.

Oat belongs to cereals rich with beta-glucane, which plays a role of prebiotic for probiotic bacteria. *Lb. reuteri, Lb. acidophilus* and *Bifidobacterium bifidum* are known to grow rapidly in an oat medium. Oat was used as a substrate for the growth of *Lb. plantarum* in order to produce a fermented beverage containing both probiotic bacteria and beta-glucane as a prebiotic. A number of the viable cells after 24 days of keeping this drink at temperature 4°C was about 10 log cfu/ml. The shelf life of this beverage containing probiotic bacteria was 21 days. Oat cereals have been applied for production of so-called yogurt with *Lb. rhamnosus*. New functional products should contain probiotic bacteria in combination with a prebiotic which is beta-glucane responsible for a decrease in cholesterol. The presence of LAB including probiotic bacteria influence beneficially on the intestinal balance of people.

Other raw material which might be a source of nutritional components for probiotic bacteria is maize. It is also known to possess a low nutritional value for people. However, some researchers have been made to confirm that some probiotics are able to survive and carry out the fermentation process (Helland et al. 2004). Previously sterilized maize porridge was separately inoculated with probiotic bacteria such as *Lb. reuteri, Lb. acidophilus* and *Lb. rhamnosus* and kept at 37°C for 24 h. The maize porridge was enriched with barley malt. The fermented products possessed similar pH values, however, these strains released lactic acid in a relatively different amount in a range of 1360 and 4000 mg/kg. *Lb. rhamnosus* was known to release the biggest amount of lactic acid. Only *Lb. reuteri* was able to use maltose but all the strains used glucose and fructose as a source of carbohydrate. *Lb. rhamnosus* was the strain which released 6 mg/kg diacetyl, which in comparison to fermented milk (1–2 mg/kg) was a significantly high amount. Certain amounts of ethanol were identified in the oat porridge fermented by *Lb. rhamnosus* and in the porridge fermented *Lb. reuteri*. It means that both strains release the dehydrogenase of alcohol. The porridge inoculated with *Lb. acidophilus* was rich with diacetyl and acetaldehyde as this strain produces alcohol dehydrogenase in a relatively small amount (GONZALEZ et al. 1994). Probiotic bacteria including *Lb. fermentum* and *Lb. plantarum* due to their ability to produce bacteriocins possess the inhibitory features against *Klebsiella pneumoniae* which was indicated in the research made by VON MOLLENDORF et al. (2006). Lactic acid fermentation causes the inhibition of *Bacillus cereus* growth. It is a widely known pathogen present in cereals, whose growth is suppressed when pH value amounts to 2.6 after 72 h.

There is one more raw material such as rice which is also a good substrate for probiotic bacteria. Apart from the fermentation process which causes a rise in amount of nutrients in cereals, there is also a process of germination increasing the quality of nutrients and bioactive compounds. During the germination process there is an increase in amount of proteins, amino

acids, sugars as well as vitamins which is vital for multiplication of probiotic bacteria (TRACHOO et al. 2006). Germinated rice grains were inoculated with *Lb. acidophilus, Lb. pentosus, Lb. plantarum* and *Lb. fermentum*. It was observed that they were able to increase a content of reducing sugars, total protein contents and vitamins.

Soybean is known to be an essential legume in the Asian diet which contains a high amount of high-quality protein (WANG et al. 2006). Soybean is popularly considered to prevent from chronic diseases like menopausal disorder, cancer, atherosclerosis, and osteoporosis (LIU et al. 2006). However, soybean possesses some disadvantages in terms of bean flavor and its content of raffinose and stachyose which lead to flatulence. The process of fermentation is often applied to enhance the digestibility of soybean and make it more tasty (HAN et al. 2001). The soymilk is known to be a good substrate for probiotic bacteria including *Lb. casei* (GARRO et al., 1999), *Lb. helveticus* (MURTI et al. 1993), *Lb. fermenti* (CHUMCHUERE and ROBINSON 1999), *Lb. fermentum* (GARRO et al. 2001, 2004), *Lb. reuteri* (TZORTZIS et al. 2004), and *Lb. acidophilus* (WANG et al. 2002, 2003, 2006). Soy beans are also known to possess phenolic compounds which show antioxidative features. It occurred that such features are significantly stronger in fermented soy products. Soymilk finds its application in the production of a cheese-like product fermented by LAB. Such cheese was produced by LIU et al. (2006). It was fermented by soy cheese bacterial starter cultures and *Lb. rhamnosus* possessing probiotic features. *Lb. rhamnosus* achieved a level of $10^8$–$10^9$ cfu/ml after 6 h of fermentation at 10°C. 30 days of keeping at 10°C leaded to a little reduction of pH value and a slight decrease in a number of viable cells. The numbers of the viable cells of *Lb. rhamnosus* and the starters amounted to $10^7$ and $10^6$. It can be concluded that *Lb. rhamnosus* was able to use soybean oligosaccharides constituting a carbon source.

It is commonly known that cow milk does not constitute a substrate in which probiotic bacteria multiply fast in comparison to soymilk (CHAMPAGNE et al. 2005). It was observed that some probiotics were able to grow faster with yogurt cultures in soymilk. FARNWORTH et al. (2007) added a combination of yoghurt starters consisting of *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* to both cow milk or soymilk containing either the probiotic bacteria *Lb. rhamnosus, Lb. johnsonii* or human derived *Bifidobacteria*. After the incubation at 41°C pH of soymilk dropped faster than in case of cow milk. It meant that in soymilk there was a higher amount of organic acids in comparison to cow milk. The presence of probiotic *Lb. rhamnosus* and *Lb. johnsonii* did not influence the growth rates of the yogurt starters. *Lb. delbrueckii* subsp. *bulgaricus* did not multiply fast in soymilk at the beginning. However, after 4 h of fermentation it started growing rapidly. It was a result of the reduction of pH value which enhanced the growth of lactobacilli. Both yoghurt starters possess the beneficial influence on themselves. *Lb. delbrueckii* subsp. *bulgaricus* is known to have the proteolytic activity, and releases free amino acids which enhance the growth of non-proteolytic bacteria.

## Meat products

Meat is considered to be an ideal substrate for probiotic bacteria. Meat prevents LAB from the lethal action of bile. It is particularly used for the production of the different dry sausages made by the fermentation without heating. Minced meat is put in casings and is left to ferment and mature (LEROY et al. 2006). Meat is the natural environment for LAB, Gram-positive, catalasepositive cocci, moulds and yeasts which start growing first (LEBERT et al. 2007). Meat in order to be fermented in a controlled way should be inoculated with a starter culture containing of selected LAB. Such microorganisms are facultative heterofermentative strains which are able to transform hexoses including glucose and lactose into lactic acid. When the glucose content is insufficient in meat, it is added in amount of 0.4–0.7% to minced meat. LAB strains which are commonly applied as starters include *Lb. casei, Lb. curvatus, Lb. pentosus, Lb. plantarum, Lb. sakei, Pediococcus acidilactici* and *P. pentosaceus*. Such functional strains allow to achieve the product which is tastier, safer and healthier (AMOR and MAYO 2007). It is known that probiotic bacteria do not grow eagerly in fermented products. In order to guarantee a proper number of probiotic bacteria in meat products, the encapsulation is used. *Lb. reuteri* and *B. longum* are alginate-microencapsulated (MUTHUKUMARASAMY and HOLLEY 2006, 2007). ARIHARA et al. (1998) indicated that LAB strains including *Lb. acidophilus, Lb. crispatus, Lb. amylovorus, Lb. gallinarum, Lb. gasseri,* and *Lb. johnsonii* should be applied in the meat fermentation to increase the safety of product. *Lb. rhamnosus* and *Lb. paracasei* subsp. *paracasei* are suitable for meat fermentation.

## Conclusion

The fermented dairy products are commonly believed to constitute the best source of probiotic bacteria due to their high nutritional value. However, non-dairy products such as vegetables, different cereals, soy and meat also constitute good substrates for the growth of very demanding probiotic bacteria. Probiotic bacteria are encouraged by the starter cultures to grow. There is a growing need to make functional probiotic products using not only milk, but also some other matrices. They have proved to possess the beneficial features for people. Despite the fact that there is a big variety of probiotic products in the market, it is still very little known about the identity and the source of some probiotic strains. As probiotic products are considered to be beneficial for human health, more researches should be carried out to confirm such features.

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