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APPLICATIONS OF PROBIOTIC BACTERIA TO THE VEGETABLE PICKLE PRODUCTS

REYHAN IRKIN[*] and GAMZE EMMUN SONGUN[a]

Balıkesir University, Susurluk Vocational School, Susurluk, TR10600, BALIKESIR, TURKEY
[a]Balıkesir University, Art and Science Faculty, Biology Dept., TR10100, BALIKESIR, TURKEY

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ABSTRACT

The consumption of live lactic acid bacteria (LAB) included in lactic acid fermented foods has been a regular part of the food intake of humans for a long time. Lactic acid fermentation is the simplest and often the safest way of preserving food. In the case of fermented foods, LAB contributes to the flavour, texture, and, in many cases, to the nutritional value of the food products. Between the LAB "probiotic bacteria" can be colonize and proliferate in the intestinal tract of humans and animals to prevent the growth of intestinal pathogens. In recent years, non-dairy-based probiotic products have increased for vegetarians or consumers. Researchers have shown that LAB can decrease the pathogen numbers in vegetable products and they can develop the immune system of the hosts. They can decrease the total cholesterol level in the blood; also they prevent irritable bowel syndrome (IBS) disorders. *Lactobacillus plantarum*, *L. delbrueckii*, *L. acidophilus*, *L. casei*, *L. lactis*, *L. buchneri* could be used as probiotic cultures for production of healthy products. In this review some new researches and applications about the probiotic vegetable pickles are summarized.

Key words: Lactobacillus, Probiotic bacteria, Vegetable pickle product.

INTRODUCTION

Vegetables are good sources of natural antioxidants such as carotenoids, vitamins, flavonoids, other phenolic compounds, minerals and dietary fibers[1,2]. One of the most effective ways of conserving perishable vegetables is fermentation. Historically various vegetables such as cabbage, cucumbers, radish, olives, carrot, green tomatoes, green peppers have been subjected to lactic fermentation. During the process traditionally a spontaneous fermentation process occurs. Generally, the lactic acid bacteria isolated from the spontaneous pickle fermentation are *Lactobacillus plantarum*, *L. brevis*, *Leuconostoc mesentereoides*, *Pediococcus pentosaceus* and *Enterococcus faecalis*[3-5].

Nowadays, the consumer pays a lot of attention to the relation between food and health. As a consequence, the market for foods with health-promoting properties, so called functional foods, has shown a remarkable growth over the last few years. In addition, food additives usage is known as unnatural and unsafe. But, additives are needed to preserve food products from spoilage and to improve the organoleptic properties. The demand for a reduced use of additives and processing seems contradictory with the market preference for products that are fresh, safe, tasty, low in sugar, fat and salt and easy to prepare. LAB is

Available online at www.sadgurupublications.com
[*]Author for correspondence; E-mail: rirkin@hotmail.com, reyhan@balikesir.edu.tr

called probiotic strains. Because they are able to produce antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, useful enzymes, or nutraceuticals and with their health promoting properties[6].

LAB produces several antimicrobials, including organic acids (lactic, acetic, formic, phenyllactic, caproic acids) carbondioxide, hydrogen peroxide, diacetyl, ethanol, bacteriocins, reuterinand reutericyclin and they can prevent mould spoilage. Chemical food additives such as propionic acid, sorbic acid and benzoic acid are commonly applied in pickle preservation. So probiotic LAB can be used in vegetable fermentation technology and in pickle industry as an alternative to the chemical preservatives[7].

Application of probiotic LAB to pickle industry represents a way of replacing chemical additives by natural compounds, at the same time providing the consumer with new, attractive and healthy food products. In this review, results of the some new researches about the probiotic LAB applications of vegetable products were summarized.

LAB's work

Lactobacilli are used as starters or complementary cultures for several varieties of foods. They cause rapid pH decrease in the raw material through the production of lactic acid as the main catabolic product and aroma compounds and bacteriocins[8].

Lactic acid production during fermentation process is commonly safe. Lactic acid fermentations include those in which the fermentable sugars are converted to lactic acid by organisms such as *Lc. mesenteroides*, *L. brevis*, *L. plantarum*, *L. bulgaricus*, *L. acidophilus* etc. Vegetable foods and vegetable/ fish/shrimp mixtures are preserved around the world by lactic acid fermentation[9]. Sauerkraut is a good example for the classic lactic acid/vegetable fermentation. Lactic acid bacteria develop in a sequence. In sauerkraut fermentation first, *Lc. mesenteroides* grow producing lactic acid, acetic acid and CO_2, which flushes out any residual oxygen making the fermentation anaerobic. Then *L. brevis* grow producing more acid. Finally, *L. plantarum* grow producing still more lactic acid and lowering the pH to below 4.0. At this pH and under anaerobic conditions, the cabbage or other vegetables will be preserved for long periods of time[10].

Pathogens

Strains from isolated from all fermented vegetables show antimicrobial activities against a number of potentially pathogenic gram-negative and gram-positive bacteria. This indicates that functional LAB can reduce the number of undesired microorganisms in vegetable products[11]. Especially, fermented raw vegetables cannot be pasteurized and they can have high microbial load, vegetables can act as a vector in transporting pathogenic bacteria from the farm. Applications of protective cultures and co-cultures are considered as additional safety factors warranting the microbiological stability of the resulting foods reducing risks of growth and survival of food-borne pathogens and food spoilage organisms[12-15]. Bacterial pathogens have been reported on fresh cucumbers and other vegetables used for commercial fermentation. The food and drug administration currently has a 5-log reduction standard for *Escherichia coli* O157:H7 and other vegetative pathogens in acidified pickle products. Breidt and Caldwell[16] made an investigation and due to that investigation the 5-log reduction times for *E. coli* O157:H7 strains in the commercial brines were found to be positively correlated with brine pH, and ranged from 3 to 24 d for pH values of 3.2 to 4.6, respectively. In a laboratory cucumber juice medium that had been previously fermented with *L. plantarum* or *Lc. mesenteroides* (pH 3.9), a 5-log reduction was achieved within 1 to 16 d depending on pH, acid concentration, and temperature. During competitive growth at 30^{o}C in the presence of *L. plantarum* or *L. mesenteroides* in cucumber juice, *E. coli* O157:H7 cell numbers were reduced to below the level of detection limit within 2 to 3 d.

In some studies, it was shown that *L. sake* C2 can produce a new bacteriocin (sakacin C2), which was isolated from traditional Chinese fermented cabbage. The antimicrobial spectrum of sakacin C2 is different from other bacteriocin and it has inhibitory activity against not only many gram-positive but also some gram-negative pathogen bacteria. Therefore it was concluded this strain may have good applications for producing functional fermented foods[17].

Effects on health

Some LAB strains can be used to improve the gut performance and reduce the pathogen invasion. Further more certain LAB strains have some other beneficial effects, such as developing of the immune system of the human hosts[18-19].

In a small randomized, placebo controlled and double-blind study on men with slightly elevated cholesterol levels, it was shown that the concentrations of total cholesterol and of low-density lipoprotein (LDL) cholesterol were decreased after consumption of *L. plantarum* 299v in a drink. The study included 30 individuals divided into two groups, where the treatment group consumed 200 mL drink (rose hip), containing 5×10^7 CFU/mL, for 6 weeks, and the placebo group consumed the drink without lactobacilli. The fall in cholesterol level was small but statistically significant[20].

Irritable bowel syndrome (IBS) is a general problem of people; however why and how it is occurring is unknown. It is a collection of disorders causing similar symptoms of abdominal pain, diarrhea, constipation, or variability of bowel habit. *L. plantarum* 299v in the fruit drink ProViva (rose hip) was administrated to patients with IBS in two double-blind, placebo-controlled studies, one in Poland[21] and one in Sweden[22]. In both studies, the patients were divided into two groups: one was given *L. plantarum* 299v and the other a similar rose hip drink without *L. plantarum* 299v (placebo). In the Polish study, that the magnitude of several of the IBS symptoms decreased in the *L. plantarum* group, and a higher proportion of the patients were free from symptoms in the treatment group than in the placebo group[21]. In the Swedish study, *L. plantarum* 299v significantly decreased the subjective bloating experienced during the treatment period. Pain was also significantly reduced in both the treatment group and in the placebo group, but the decrease was more rapid and more pronounced in the *L. plantarum* group[22].

Chiu et al.[18] demonstrated two strains of *L. plantarum* and *P. pentosaceus* from pickled vegetables, which can be used as probiotics to prevent the *Salmonella* invasion in animal metabolism.

Beside of antimicrobial effects of probiotic bacteria another health advantage of them was shown that specific lactic acid strains of the *Lactobacillus* genus can effectively prevent biogenic amines formation in the sauerkraut production[11-12].

Some researches about probiotic vegetable pickles

Although LAB is the principal microorganisms responsible for the natural fermentation of vegetables, the indigenous LAB flora varies as a function of the quality of the raw material, temperature and harvesting conditions. Strains of species belonging to Lactobacillus (*L. plantarum*) and Leuconostoc (*L. mesenteroides*) are the most common bacteria in natural vegetable lactic acid fermentation but *L. paracasei/casei*, *L. delbrueckii* and *L. brevis* have been reported as well. It has been suggested that besides growing well on vegetables juices as the sole substrate in lactic acid fermentation, *Leuconostoc* species selectively promote some interesting bacterial species such as *Lactobacilli* and *Bifidobacteria*, thus equilibrating intestinal microflora due to the synthesis of dextransucrase[23].

The viability and activity of probiotic bacteria during preparation and storage is very important for their industrial application. They can be added to probiotic products as fresh or lyophilized cells. High population levels, between 10^6-10^8 microbial cells/mL should be present in probiotic products. It should be also explained that during food processing bacteria subjected to stress conditions such as freezing, drying and concentration stress and these are also decrease the viability of LAB[24].

Yoon et al.[25] researched red beet juice fermentation with *L. acidophilus*, *L. casei*, *L. delbrueckii* and *L. plantarum* in their study. They found that *L. acidophilus* in fermented beet juice could be remained at 10^6-10^8 cfu/mL after 4 weeks of cold storage and the others lost their viability. Between the LAB strains used in red beet juice fermentation process *L. paracasei* 0923 was found the most promising strain in Czyowska et al.[26] research.

In another study Yoon et al.[27] determined suitability of cabbage as a raw material for production of probiotic cabbage juice by *L. plantarum C3*, *L. casei A4* and *L. delbrueckii D7* in their study. Viable counts of *L. plantarum* and *L. delbrueckii* were still 10^7 and 10^5 at 4°C respectively after the 4 weeks. But *L. casei* did not survive the low pH and high acidity conditions. Also they found the viable cell counts of the four lactic acid bacteria (*L. acidophilus* LA39, *L. plantarum* C3, *L. casei* A4, and *L. delbrueckii* D7) in the fermented tomato juice ranged from 10^6 to 10^8 CFU/mL after 4 weeks of cold storage at 4°C in a different study[28].

In another study, it was found that all tested *Bifidobacterium* strains were found to be capable of growing well on pure pasteurized carrot juice without nutrient supplementation[29].

Kimchi is a Korean traditional fermented vegetable mainly Chinese cabbage and radish. Lee and Lee[30] demonstrated that survival of *L. plantarum* PL62 during fermentation suggested a functional probiotic can be added to various fermented foods making functional foods such as kimchi and pickle.

Beganovic et al.[31] showed *L. plantarum* L4, *L. mesenteroides* LMG 7954 strains including in sauerkraut pickles are considered as probiotic products because probiotic cells in final product was determined higher than 10^6 CFU/g.

Settani and Corsetti[32] considered a developed paired culture system for sauerkraut, consisting of the nisin resistant *Lc. mesenteroides NCK 293* and the nisin producing *L. lactis NCK 401*. Nisin level was the constant during 12 days in the product. But the cell populations and nisin levels could be altered by changing the initial ratios of *Lc. mesenteriodes* and *L. lactis*.

Tursu is a traditional fermented Turkish pickle made of vegetables such as cabbage, cucumber, carrot, beet, pepper, turnip, eggplant and beans. It was determined that *L. plantarum* as a starter culture into the tursu improved taste and beneficial properties of the product. In the research it was also stated that although *P. pentosaceus*, *Lc. mesenteroides* can be used of fermented vegetables. However, *L. plantarum* is more useful and adaptive microorganism. It was determined that *L. plantarum* NCULI005 has conjugated linoleic acid producing capacity, too[33].

CONCLUSION

During this review, we indicated that probiotic fermentation of vegetables are healthy, safety and may be fermentation is the easiest way of preserving foods that depend on traditions; which are alive for centuries, and scientific investigations. Fermented vegetables and pickles could serve as a healthy beverage for vegetarians and lactose-allergic consumers.

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