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Review

The antioxidant activity of Lactic acid bacteria and probiotics: a review

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ABSTRACT

Probiotics and Lactic Acid Bacteria play important roles such as the production of antimicrobial compounds and other metabolites. So they have positive effects on human health. When reactive oxygen species generated in excess or cellular defenses are deficient, biomolecules can be damaged by the oxidative stress process. Various studies have shown that the best way to protect the human body from the effects of oxidation reactions is to avoid them, which can be accomplished by using antioxidants. Due to the damages of synthetic antioxidants, their usage has been discussed. Nowadays natural antioxidants derived from bio-resources have recently gained a lot of attention as a potential replacement for synthetic antioxidants. Probiotic bacteria are thought to defend against oxidative stress by restoring the gut microbiota, according to hypothesis of some scientists. This type of microorganisms indicated their antioxidant activity by producing and increasing antioxidant enzymes, production of secondary metabolites, small hydrolyzed peptides in food, resistance to the presence of hydrogen peroxide, and production of intracellular and extracellular compounds such as Exopolysaccharides. Also, they have shown their positive effect on in vivo models. In conclusion, according to the results of studies, lactic acid bacteria and probiotics are significant sources of natural antioxidants. Therefore, they have important research value and market development potential. Also, it should be noted that the mechanism of antioxidant activity of this group of microorganisms has not been fully investigated, this requires further research.

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1. Introduction

According to the definition of FAO/WHO, probiotics can be explained as: "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (1).

In the dairy industry, probiotics play important roles such as the production of antimicrobial compounds and other metabolites, anticarcinogenic and antimutagenic properties, controlling gastrointestinal infections, and immune system stimulation (2,3).

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During the process of cellular metabolism, some reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) are produced. They play important roles in cell signaling, apoptosis, gene expression, and ion transportation. However, when these ROS molecules are generated in excess or cellular defenses are deficient, biomolecules including protein, lipid, and nucleic acids can be damaged by the oxidative stress process (4).

The superoxide radical is a powerful oxidant that can interfere with any biomolecule in liver cells (5). Some damages can cause cancer, allergies, cardiovascular, atherosclerosis, inflammation, HIV progression to AIDS, and age-related degenerative diseases like Alzheimer's and Parkinson's disease (4,6,7). Actually, oxidative stress is a significant predictor of morbidity and mortality. Although increased inflammation and oxidative stress are predictors of mortality in hemodialysis patients, long-term elevated oxidative stress, rather than inflammation, is a major risk factor for atherosclerosis and cardiovascular mortality in these patients (8).

Various studies have shown that the best way to protect the human body from the effects of oxidation reactions is to avoid them, which can be accomplished by using antioxidants. Antioxidant compounds are thought to be able to slow or avoid the oxidation process, potentially protecting the human body from oxidative damages. Antioxidants are the first and most important defense system against ROS molecules, which are classified into two groups: natural and synthetic. Actually, antioxidants compounds remove free radical chain reactions by dissolving them and also prevent the formation of reactive oxidants (76).

Most living organisms have an enzymatic or non-enzymatic antioxidant defense system that protects them against oxidation reactions. However, natural antioxidants are not enough to protect living organisms from the damages of the oxidation reaction, so the usage of antioxidants is highly recommended by scientists and doctors. Some synthetic antioxidants such as Butylated hydroxytoluene (BHT) and Butylated hydroxyanisole (BHA) are widely used, but due to the damages of synthetic antioxidants, the usage of them has been discussed. Nowadays natural antioxidants derived from bio-resources have recently gained a lot of attention as a potential replacement for synthetic antioxidants (4).

Most probiotics are classified in the category of lactic acid-producing bacteria (LAB), this group of microorganisms is widely found in nature and also plays an important role in the food industry, especially in dairy products. Species with essential biological functions such as antioxidant properties have recently been discovered, in addition to their role in the manufacture of various fermented products and their beneficial effects on human health. Metabolic activities of LAB and Probiotics may have demonstrated an antioxidant effect by scavenging oxidant compounds or preventing their generation into the intestinal tract (9). Of course, when using probiotic bacteria, attention should be paid to the various dimensions that are assessed in the process of selecting probiotic microorganisms, such as technical characteristics, functional characteristics, and protection (78).

This paper, it will be argued about the results of studies in various fields. This study aims to gather some information about one of the most important properties of LAB and probiotic bacteria, antioxidant activity.

2. Methods for measuring the antioxidant activity

Several methods exist to measure the antioxidant activity. In various studies, these types of methods have used such as:

1. The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity (freshly prepared DPPH solution).
2. Total antioxidant activity against ascorbic acid (TAA_{AA}) and linolenic acid oxidation (TAA_{LA}) which uses the thiobarbituric acid (TBA) method.
3. Superoxide dismutase (SOD) activity, based on the ability of tested samples to inhibit the forming of red formazan nitroblue tetrazolium produced by the reaction of xanthine and xanthine oxidase and Glutathione (GSH) content, was performed using Ellman’s reagent 5,5-dithiobis-2-nitrobenzoic acid (DTNB).

The results of many studies indicate that the antioxidant properties measured according to the methods that are commonly used for probiotics and LAB, which are strains specific features (10,11,12,13,14).

3. Antioxidant activity and the protective effects against oxidative stress

Many studies have documented that one of the probiotics' beneficial effects is a defense against oxidative stress in humans. Probiotic bacteria are thought to defend against oxidative stress by restoring the gut microbiota, according to the hypothesis of some scientists (15,16). The results of various studies have shown that the usage of certain species of Lactobacillus and Bifidobacterium can be very effective to increase the level of antioxidants. This may be due to the production of various compounds by this group of microorganisms (17,18,19). The interpretation of the results of scientist’s studies is as follows:

4. Enzymes and antioxidant defenses

One of the most essential compounds in chemical reactions is enzymes. According to several reports, some LAB species may generate antioxidant substances such as superoxide dismutase (SOD), glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GP_X), glutathione S-transferase (GSTs), catalase (CAT), NADH oxidase, NADH peroxidase, and feruloyl esterase. These substances are thought to be essential enzymes that combat oxidative stress (20,21). According to findings of Spyropoulos et al., (2011) probiotic bacteria can help the host's antioxidant defenses by producing and releasing enzymes and vitamins that are then absorbed and distributed throughout the body (22). Similarly, Wang et al., (2009) reported that by increasing antioxidant enzymes like SOD and GPx, *L. fermentum* is able to keep pigs growing healthy (23). In a study by Zhang et al., (2010) the antioxidant ability of L. Casei was found to be beneficial in hyperlipidaemic rats, as evidenced by an increase in antioxidant enzymes such as SOD and GPx activity (24). As a result, these findings indicated that the usage of LAB can improve the synthesis of important antioxidant enzymes, which could help to avoid pathological oxygen radical concentrations or improve biochemical changes.

5. Cell surface compounds and antioxidant activity

Some LAB strains' antioxidant activity can also be due to their development of cell-surface compounds, such as extracellular polysaccharides derived from bacterial cell surfaces (25-27). A study by Li et al., (2012) confirmed that the cell-surface proteins or polysaccharides of *L. plantarum* C88 were involved in the antioxidant activity of this strain. When these cell surface compounds were removed, a significant decrease of the DPPH free radical scavenging capacity was observed. In addition, the findings of this study showed that administering *L. plantarum* C88 to D-galactose-treated mice increased their levels of SOD and GPx (4). Also, many studies have been working on the antioxidant activity of LAB and probiotic bacteria through their Exopolysaccharides (EPS). Actually, Cell-surface proteins, EPS, and lipoteichoic acids, which are capable of chelating iron metal and scavenging superoxide radicals, have been credited with LAB's antioxidant operation (12). In a study by kodali et al., (2008) a probiotic strain showed high antioxidant activity. The β-carotene-linoleate model system, in which one hydrogen atom of linoleic acid is removed, leaving a free radical ready to strike β-carotene molecules, was used to calculate total antioxidant activity. The rate of inhibition of linoleic acid oxidation by EPS and vitamin C, respectively, was found to be 74% and 92%. As a result, the antioxidant activity of the EPS provided by this strain was high. When the antioxidant activity of EPS was associated with its concentration, the antioxidant activity increased up to 500 g/mL and then remained constant. At a concentration of 500 g/mL, EPS demonstrated significant hydroxyl radical scavenging activity (62.05%), but no further activity was observed.

6. Effect of bacterial growth on antioxidant activity

Some studies have reported that bacterial growth has an effect on antioxidant activity. In a study by Virtanen et al., (2007) the antioxidant activity of 25 LAB strains was tested. Many of the strains were able to scavenge radicals. The calculated activities varied depending on the strain, with inhibition ranging from 3% to 53%.Generally, *Lactobacillus* and *Leuconostoc* exhibited higher antioxidant activity than *Lactococcus*. Furthermore, the antioxidant activity of bacteria differs at different stages of growth, and the production of lipid peroxidation inhibitory activity varies among the strains, according to the findings of this research. The formation of lipid peroxidation inhibitory activity was linked to bacterial growth in *Leuconostoc cremoris* and *Lactobacillus jensenii*. (19). Tang et al., (2018) also found that the logarithmic phase of *Lactobacillus plantarum* MA2 has higher antioxidant activity than the stationary phase. This may be due to the development of secondary metabolites and other compounds (29).

7. Lactobacillus species and antioxidant activity

Various researches have been worked on *Lactobacillus* species up to now. In a study by Li et al., (2012) Eleven *Lactobacillus plantarum* strains isolated from traditional

Chinese fermented food were investigated in vitro and in vivo models.

In the analysis of the behavior of *L. plantarum* C88 (at a dose of 10^{10} CFU/ml) against hydroxyl and DPPH free radicals, and the resistance to hydrogen peroxide, this specie showed the highest hydroxyl radical and DPPH scavenging activities, with inhibition rates of 44.31% and 53.05%, respectively. Resistance of intact cells to hydrogen peroxide was also found in all strains. *L. plantarum* C88 was the most resistant strain against hydrogen peroxide. Furthermore, the results which are obtained from in vivo model (rats) showed that the usage of *L. plantarum* C88 significantly improves the number of compounds with antioxidant properties such as SOD and GPx (4). *Lactobacillus fermentum* ME-3 increased the GSH redox ratio in blood sera in a study by Mikelsaar & Zilmer (2009) (14), and *Lactobacillus casei* decreased lipid peroxidation in a study by Zhang et al., (2010), leading to improved lipid metabolism in the blood (24). Ahotupa, Saxelin, & Korpela (1996) have shown that *Lactobacillus rhamnosus* GG inhibits lipid peroxidation due to iron chelation and superoxide radical scavenging ability (30). Also, Achuthan et al., (2012) reported the resistance of a total of 39 *Lactobacillus* cultures to ROS. *Lactobacillus* spp. S3 and *Lactobacillus plantarum* 55 had the highest overall antioxidant activity of the 39 isolates, with 77.85% and 56.1% inhibition of linolenic acid oxidation, respectively (31). Kapila & Sinha (2006) used Microsome-Thiobarbituric acid (MS-TBA) and the linoleic acid peroxidation process to investigate the antioxidant capacity of 13 *Lactobacillus* strains. Maximum antioxidant capacity in terms of percent inhibition of oxidation was expressed in *L. casei* ssp. *casei* 19 (76.82%) (32).

In addition, Kullisaar et al., (2002) isolated two antioxidant *L. fermentum* strains from a healthy child's intestinal microflora. These strains were found to have a high antioxidant activity. Furthermore, they were able to live longer in the presence of hydroxyl radicals and express SOD (12). In other studies, *Lactobacillus* strains such as *L. brevis* BJ20 and *L. plantarum* 7FM10 have also been shown to have DPPH anti-radical activity (4,33,34). Nyanzi et al., (2015) used the highest probiotic bacteria extract concentration (20 mg/mL) in their research. The DPPH scavenging activities of *L. acidophilus*, *L. rhamnosus*, and *L. casei* strains were 77.9 – 86.1%, 45.71 – 86.4%, and 36.92 – 45.80%, respectively. Since they each had the lowest IC_{50} of 4.24 - 4.31 mg/mL, extracts from *L. acidophilus* strains had the highest antioxidant activity. The IC_{50} of all extracts from the *L. rhamnosus* strains tested ranged from 6.37 to 8.97 mg/mL. *L. casei* extracts were the least effective at reducing DPPH free radicals, with IC_{50} values ranging from 9.35 to 24.07 mg/mL. Antioxidant components were found in varying amounts in extracts from freeze-dried probiotic cells, according to this report (35).

Furthermore, Lactobacillus rhamnosus had a significant impact on the DPPH radical scavenging behavior of cheeses during the ripening period (P>0.05), which was significantly higher than the control group (36). Thus, the antioxidant activity is the basis for the increased resistance of some *Lactobacillus* strains to toxic oxidative compounds and helps them to serve as defensive components in the intestinal microbial ecosystem (12).

It should be mentioned that the antioxidant activity depends on bacterial strain. This means that not all species of a genus have antioxidant properties. The antioxidant activity of various *Lactobacillus* strains derived from the human oral cavity was studied, and it was discovered that both intact cells and clear cell lysates of various *Lactobacillus* species could exhibit antioxidant properties; however, there was a wide range of individual DPPH, TAA_{LA}, SOD, and GSH properties within and between Lactobacillus species. Except for *L. oris* and *L. gasseri*, all of the *Lactobacillus* strains studied performed well in all antioxidant parameters. Their findings revealed that antioxidant properties vary by strain (37). According to a hypothesis proposed by Amaretti et al., (2013), some probiotic strains can contribute to the enhancement of cellular antioxidant defenses in the host (38). Also, the results of others were in accordance with previous findings where only certain *Lactobacillus* strains could exhibit antioxidant activities (4,31,39,40). Lin & Chang (2000) also found that some intestinal LAB, inhibiting linoleic acid oxidation, revealed significant antioxidant activity (in the case of *L. acidophilus* ATCC 4356 an antioxidant capacity was 28–45%). In fact, their results indicated that antioxidant properties are strain-specific (41).

8. Fermented foods and antioxidant activity

In studies conducted by scientists, different bacterial species isolated from food and their antioxidant properties has been worked. In a study by Songisepp et al., (2004) *L. fermentum* ME-3 was inoculated with a dose of 10^9 cfu/mL during the production process of a new cheese called Pikantne.

When this strain was added into cheese, the ME-3 cells are likely to be stressed by the cheese-making process, as shown by a decrease in cell counts, TAA_{AA}, and antimicrobial properties. It's possible that ME-3's functional properties need time to adjust to the unusual cheese environment. The cells that survived from cheese making began to multiply almost in parallel with TAA_{AA} formation, and after two months (66 days after cheese preparation), they had reached the same TAA_{AA} levels as the original ME-3 culture. Nevertheless, further clinical trials are required to prove the health benefits of probiotic bacteria in cheese. Their findings revealed that their product (open-textured and soft cheese) was a good probiotic delivery vehicle for this strain and could be used as a new probiotic cheese supplement (42). The results of a study by Sun, Chou & Yu (2009) are consistent with the observations of Lee et al., (2010) who found that *Lactobacillus brevis* BJ20 strain isolated from traditional Korean fermented food showed significant scavenging potency in the range of 87.7- 92.8%. Also, *Leuconostoc mesenteriodes* and *Lactobacillus plantarum* which were involved in the fermentation of Chinese cabbage exhibited a maximum of 96.99% scavenging of DPPH radical (33,43).

In addition, from a type of Korean food called kimchi, *L. lactis* KC24 strain was isolated and showed 35.15% of antioxidant activity in a β-carotene assay (44). In another study, the probiotic and synbiotic yogurt samples had a high antioxidant potential when compared to the control group. Moreover, the total phenolic content in synbiotic yogurt samples was higher ($p<0.05$) than the control group.

They reported that the increased total phenolic content in synbiotic yogurt could be due to the fermentative activity of probiotic bacteria. Also, a significant correlation was observed between the total phenolic content and the antioxidant activity (FRAP assay; r = 0.853) (45). Actually, phenolic compounds were the main contributors toward the antioxidant capacity (46). It has also been reported that yogurt starter bacteria including *Streptococcus salvarius* ssp. *thermophilus* ATCC 19258 and *Lactobacillus delbrueckii* ssp. *bulgaricus* ATCC 11842 have a specific effect on oxidative stress (47). Milk and dairy products are antioxidant-rich foods that can help consumers' oxidative defenses while also avoiding lipid peroxidation (77). In various studies, scientists have concluded that fermentation has a positive effect on increasing antioxidant activity. Also, fermentation type has an important role in antioxidant activity (48). Antioxidant activity was measured by the ability of the *Pediococcus pentocaseus* in fermented milk to scavenge the free radicals. Based on ANOVA results, fermented cow, goat, and camel milk had significantly higher antioxidant activity than their respective unfermented ones (49).

Consumption of fermented milk containing *L. fermentum* ME-3 has been shown to have a beneficial impact on human health in healthy volunteers, with antioxidant activity being the explanation for this (50). According to a report, bacterial cells, metabolic substances liberated from cells, or hydrolyzed milk components are the cause of fermented milk's increased antioxidant activity.

Intestinal strains including *Bifidobacterium longum* and *Lactobacillus acidophilus* have been shown to have antioxidant properties, preventing linoleic acid peroxidation and scavenging free radicals. Both radical scavenging activity and inhibited lipid peroxidation increased during the fermentation period. Also, they reported that the radical scavenging activity is more dependent on certain specific proteolytic enzymes of a bacterial strain than a proteolytic state of the fermented product (51). Moreover, Hernández-Ledesma et al., (2005) found a moderate ABTS radical scavenging capacity in commercial fermented milk from Europe (52). Regarding the expression of antioxidant activity in fermented milk Nishino et al., (2000) reported that increased radical scavenging activity was due to the protein peptides present in the fermented milk (53). Actually, in foods containing probiotic bacteria, the development of bioactive peptides has been considered an effective mode of antioxidant activity (54). Also, the peptide hydrolyzed from fermentation could be responsible for minimizing oxidative stress when analyzed for their antioxidant activity by the β-Carotene bleaching assay (55). The mechanism of this action was explained by Abadía-García et al., (2013) and Cumby et al., (2008), Proteolysis

may result in the formation of various antioxidant peptides, which would increase the antioxidant activity of Cheddar cheese, if the ripening period of the cheese was extended. The ability of samples to donate electrons or protons is directly related to their reducing power, which is determined by peptide cleavages (56,57).

In a study by Timón et al., (2014), Casein hydrolysates showed high antioxidant activity and the ability to inhibit free radicals, the presence of peptides was the main reason for this property (58). Also, in another study, it has been observed that casein digestion liberate small peptides with radical scavenging activity (59). The antioxidant activity of whey from skimmed milk fermented with *Lactobacillus* strains was tested in other studies. Their findings clearly show that antioxidant activity develops in fermented milk whey. The whey from milk fermented with the strain of *L. brevis* isolated from a dairy product had the highest degree of antioxidant activity, according to the findings of these studies. In comparison to the other non-dairy isolates, this is likely due to the strain's greater adaptation to the milk substrate. Extracellular metabolites, hydrolyzed milk components, and/or cell lysis products may be responsible for the observed antioxidant activities when the cells and casein were extracted by centrifugation (19,41,49,60,61). According to the results of these studies, radical scavenging activities of fermented milk suggest that they could be used as a natural antioxidant supplements for improving human health.

9. Resistance to Hydrogen Peroxide and antioxidant activity

Chooruk et al., (2017) discovered that strains with high DPPH and TAALA activities were able to withstand oxidative stress better than strains with low antioxidant activity. Their findings revealed that strains of *L. fermentum*, *L. paracasei*, and *L. rhamnosus* with high DPPH and TAA_{LA} activities could survive (> 60%) after being incubated for 8 h in 1.0 mmol l^{-1} hydrogen peroxide and 1 h in 1.0 mmol l^{-1} hydroxyl radicals, respectively. The strains of *L. salivarius*, *L. oris*, and *L. gasseri*, on the other hand, did not survive. It is worth noting that the mechanism of resistance to hydrogen peroxide in different strains of *Lactobacillus* is still unknown. (37). In other studies, it was shown that *L. casei* KCTC 3260 and *L. rhamnosus* GG survived after 8 h of 1.0 mmol l^{-1} hydrogen peroxide incubation (62) while *L. fermentum* E-3 and E-8 survived for 180 and 150 min, respectively (12). Li et al. (2012) found that L. plantarum C88, C10, and K25 were resistant to hydrogen peroxide, while L. plantarum S5-2, S5-6, and S7-2 were sensitive (4). It may have been a coincidence, but the *Lactobacillus species* that could generate hydrogen peroxide on tetramethylbenzidine-plus agar plates were thought to have strong antioxidant activity. The strains may be able to balance oxidative stress and antioxidant compounds, but further research is needed to fully understand the mechanisms (63). Two antioxidant strains of *L. fermentum* were isolated from a healthy child's intestinal microflora. They investigated the resistance of two highly antioxidant *L. fermentum* strains, E-3 and E-18, to different ROS-rich environments in this research. They compared the survival of E-3 and E-18 with the non-antioxidant strain of *L. fermentum* E-338-1-1 and *Salmonella typhimurium*. Their main findings were that the antioxidant strains E-3 and E-18 (when compared to the non-antioxidant strain E-338-1-1) survived longer in a 0.4 mmol l^{-1}

hydrogen peroxide environment, were able to multiply in a medium with abundant superoxide radicals, and had improved resistance to hydroxyl radicals (12). Mishra et al., (2015) discovered that in the presence of H_2O_2 and hydroxyl radical, the survival time of the *L. rhamnosus* GG strain was substantially longer than that of non-antioxidant strains such as *L. paracasei* Fn032 and *L. plantarum* Fn001. In addition, *L. paracasei* Fn032 and *L. plantarum* Fn001 were specific for the free radical scavenging activities of their intracellular free extracts (ICFE) (63). Furthermore, scientists discovered that by accumulating GSH, *L. lactis* subsp. *cremoris* strain SK11 has greatly enhanced H_2O_2 tolerance. Antioxidants such as Mn^{2+},-SH, peptides, and amino acids also play a positive role (64).

10. Molecular Weight

Researchers discovered that molecular weight has an impact on antioxidant activity in some experiments. The increased antioxidant capacity of cheese after simulated gastrointestinal digestion, according to Liu et al., (2018), is likely to be linked to a small molecular weight polypeptide from casein with high antioxidant activity, which is degraded by pepsin, trypsin, and protease generated by probiotics (36). Also, the results of the study of Timón et al., (2014) showed the relationship between the concentration of low molecular weight peptides (<3 kDa) and antioxidant activity in cheese extracts (58). This achievement has also been described in other researches where the antioxidant activity was associated with higher peptide concentration in extracts derived from bovine casein hydrolysates and also water-soluble extracts from cheese (72,73) or different protein sources (74,75). Farvin et al., (2010) mentioned that the lower molecular weight fractions are more effective antioxidants than higher molecular weight fractions. In this study, the lower molecular fractions were examined as antioxidants in fish-oil-enriched milk. On the basis of peroxide value, tocopherol, volatiles, and sensory characteristics, the lower molecular weight fractions 3-10 kDa and ‹3 kDa showed protection against oxidation of fish oil to the same extent as caseinophosphopeptides. Their results showed that the two lower molecular weight fractions (3-10 kDa and ‹3 kDa) have compounds with high reducing power. Also, they reported that the lower molecular weight fractions 3–10 kDa and ‹3 kDa showed higher iron-chelating activity and reduction than higher molecular weight fractions (65).

11. Probiotic bacteria and antioxidant activity in vivo

Probiotic bacteria, as previously mentioned, have beneficial effects on human health. In vitro methods are used to determine each characteristic of probiotic bacteria, followed by in vivo experiments in appropriate animal models.

Following confirmation of the organism's suitability, it is incorporated into a suitable food product, and further research is performed using probiotic food to determine its effectiveness, storage stability, and sensorial and chemical properties (37). In a rat sample, probiotic supplementation was found to increase GSH synthesis in pancreatic cells and induce transcription of genes involved in GSH biosynthesis in the intestinal mucosa (66,71). Rats were fed with probiotic strains supplementation showed similar efficacy in restoring GSH levels (67). Grompone et al. (2012) developed a modern, fast, predictive, and convenient in vitro method for screening the antioxidant potential of new probiotic strains (Using the nematode *Caenorhabditis elegans* as a host). A total of 78 LAB (*Lactobacillus* and *Bifidobacterium*) strains were examined in this study. The strains used in this research were fed to *C. elegans*, and the survival rate of *C. elegans* was compared to the protective effect exerted by *E. coli* OP50 after exposure to H_2O_2-induced stress. This screening revealed that the *L. rhamnosus* strain (CNCM I-3690) has a high antioxidant capacity (68). 570 strains were screened by Kaizu et al. (1993). An assay using rat liver microsomes and thiobarbituric acid revealed that 19 of them had antioxidant activity. Seven *Lactobacillus* strains were found to inhibit oxidation activity after further investigation. The highest activity was obtained by heterofermentative *Lactobacillus* sp. SBT 2028 (69). It is worthy to mention that the obligately homofermentative *Lactobacillus* group was found to have the highest activity when compared to the other groups (48). In fact, the results of various studies showed that the antioxidant activity of *Lactobacillus* species depends on different factors such as facultative and obligatory hetero-fermentative features (70).

Conclusion

Considering the fact that the human microbiota has to be tolerant endogenous and exogenous oxidative stress, antioxidant strains, with desirable properties, maybe a promising material for both applied microbiology and scientific food industry. Probiotics are thought to play a variety of biological roles through different mechanisms, one of the most significant and debated of them is antioxidant activity. According to the results of studies, lactic acid bacteria and probiotics are a significant source of natural antioxidants. As a natural antioxidant, they have important research value and market development potential. Given that administered probiotics colonize the colon, it's possible that their overall protective effect is linked to activities occurring in the intestine, i.e., secretion of enzymes like SOD, metal-chelating activities, promotion of the production of antioxidant biomolecules such as Exopolysaccharides, showing an in vivo antioxidant and free radical scavenging activities. Furthermore, the intestinal microflora provides additional enzymatic activities which involved in the transformation of dietary compounds, so increasing the bioavailability of dietary antioxidants. Taking into consideration, the selection of specific strains and proof of their efficacy can be used to develop novel probiotic foods or supplements which can help reduce oxidative stress and related diseases.

The findings can also point to the need to develop indigenous Lactobacillus strains as food fermentation starters or as possible replacements for synthetic antioxidant food additives including BHT and BHA, which have been used for a long time and are known to be secure. Thus, scavenging properties of free radicals by food grade cultures can be useful in food manufacturing and can provide additional dietary sources of health-enhancing antioxidants.

Finally, it should be noted that the mechanism of antioxidant activity of this group of microorganisms has not been fully investigated, this requires further researches.

References

1. Hill C, Guarner F, Reid G, et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014; 11: 506-514.
2. Ott A, Hugi A, Baumgartner M, et al. Sensory investigation of yogurt flavor perception: Mutual influence of volatiles and acidity. J. Agric. Food Chem 2000; 48: 441-450.
3. Pinto SM, Clemente MDG, De abreu LR,. Behaviour of volatile compounds during the shelf life of yoghurt. Int J Dairy Technol 2009; 62: 215-223.
4. Li S, Zhao Y, Zhang L, et al. Antioxidant activity of *Lactobacillus plantarum* strains isolated from traditional Chinese fermented foods. Food Chem 2012; 135: 1914-19.
5. Halliwell B, Gutteridge JMC. Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. Arch Biochem Biophys 1986; 246: 501–514.
6. Choi CW, Kim SC, Hwang SS, et al. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Sci J. 2002; 163: 1161-1168.
7. Shen Q, Shang N, Li P. In vitro and in vivo antioxidant activity of *Bifidobacterium* animalis 01 isolated from centenarians. Curr Microbiol 2011; 62: 1097-1103.
8. Bayes B, Pastor MC, Bonal J, et al. Oxidative stress, inflammation and cardiovascular mortality in haemodialysis—role of seniority and intravenous ferrotherapy: analysis at 4 years of follow-up. Nephrol. Dial. Transplant 2006; 21: 984-990.
9. Azcárate-Peril MA, Sikes M, Bruno-Bárcena JM. The microbiota, gastrointestinal environment in prevention of colorectal cancer? Americ J Physioloic
10. Mishra O, Kovachich G. Inhibition of the autoxidation of ascorbate and norepinephrine by extracts of *Clostridium butyricum*, Megasphaera elsdenii and *Escherichia coli*. Life Sci 1984; 35: 849-854.
11. Lin MY, Yen CL. Antioxidative ability of *lactic acid bacteria*. J. Agric. Food Chem 1999; 47: 1460-1466.
12. Kullisaar T, Zilmer M, Mikelsaar M, et al. Two antioxidative *lactobacilli* strains as promising probiotics. Int J Food Microbio 2002; 72: 215-224.
13. Zanoni S, Pompei A, Cordisco L, et al. Growth kinetics on oligo- and polysaccharides and promising features of three antioxidative potential probiotic strains. J Appl Microbiol 2008; 105: 1266-76.
14. Mikelsaar M , Zilmer M. *Lactobacillus fermentum* ME-3–an antimicrobial and antioxidative probiotic. Microb Ecol Health Dis 2009; 21: 1-27.
15. Nardone G, Compare D, Liguori E, et al. Protective effects of *Lactobacillus paracasei* F19 in a rat model of oxidative and metabolic hepatic injury. Am J Physiol Gastrointest Liver Physiol 2010; 299: 669-676.
16. Forsyth CB, Farhadi A, Jakate SM, et al. *Lactobacillus GG* treatment ameliorates alcohol-

induced intestinal oxidative stress, gut leakiness, and liver injury in a rat model of alcoholic steatohepatitis. Alcohol 2009; 43: 163-172.

17. Naruszewicz M, Johansson ML, Zapolska-Downar D, et al. Effect of *Lactobacillus plantarum 299v* on cardiovascular disease risk factors in smokers. Americ J Clin Nutr 2002; 76: 1249-55.

18. Songisepp E, Kals J, Kullisaar T, et al. Evaluation of the functional efficacy of an antioxidative probiotic in healthy volunteers. Nutr J. 2005; 4: 1-10.

19. Virtanen T, Pihlanto A, Akkanen S, et al. Development of antioxidant activity in milk whey during fermentation with *lactic acid bacteria*. J. Appl Microbiol 2007; 102: 106-115.

20. Wang X, Geng X, Egashira Y, et al. Purification and characterization of a feruloyl esterase from the intestinal *bacterium Lactobacillus acidophilus*. Appl Environ Microbiol 2004; 70: 2367-2372.

21. Arasu MV, Kim DH, Kim PI, et al. In vitro antifungal, probiotic and antioxidant properties of novel *Lactobacillus plantarum K46* isolated from fermented sesame leaf. Ann Microbiol 2014; 64: 1333-46.

22. Spyropoulos BG, Misiakos EP, Fotiadis C, et al. Antioxidant properties of probiotics and their protective effects in the pathogenesis of radiation-induced enteritis and colitis. Dig Dis Sci 2011; 56: 285-294.

23. Wang A, Yi X, Yu H, et al . Free radical scavenging activity of *Lactobacillus fermentum* in vitro and its antioxidative effect on growing–finishing pigs. J Appl Microbiol 2009; 107: 1140-48.

24. Zhang Y, Du R, Wang L, et al. The antioxidative effects of probiotic Lactobacillus casei Zhang on the hyperlipidemic rats. Eur Food Res Technol 2010; 231: 151-158.

25. Pan D, Mei X. Antioxidant activity of an exopolysaccharide purified from *Lactococcus lactis subsp. lactis 12*. Carbohydr. Polym 2010; 80: 908-914.

26. Xu, Shen Q, Ding X, et al. Chemical characterization and antioxidant activity of an exopolysaccharide fraction isolated from *Bifidobacterium* animalis RH. Eur Food Res Technol 2011; 232: 231-240.

27. Yi ZJ, Fu YR, Li M, et al. Effect of LTA isolated from *bifidobacteria* on D-galactose-induced aging. Exp. Gerontol 2009; 44: 760-765.

28. Kodali VP, Sen R. Antioxidant and free radical scavenging activities of an exopolysaccharide from a *probiotic bacterium*. Biotechnol J Healthcare Nutr Technol 2008; 3: 245-251.

29. Tang W, Li C, He Z , et al. Probiotic properties and cellular antioxidant activity of *Lactobacillus plantarum* MA2 isolated from Tibetan kefir grains. Probiotics Antimicrob 2018; 10: 523-533.

30. Ahotupa M, Saxelin M, Korpela R. Antioxidative properties of *Lactobacillus GG*. Nutr 1996; 31: 51S.

31. Achuthan AA, Duary RK, Madathil A, et al. Antioxidative potential of *lactobacilli* isolated from the gut of Indian people. Mol Biol Rep 2012; 39: 7887-97.

32. Kapila S, Sinha VP. Antioxidative and hypocholesterolemic effect of *Lactobacillus casei ssp casei* (biodefensive properties of lactobacilli). Indian J Med Res 2006; 60: 361-370.

33. Lee BJ, Kim JS, Kang YM, et al. Antioxidant activity and γ-aminobutyric acid (GABA) content in sea tangle fermented by *Lactobacillus brevis BJ20*

isolated from traditional fermented foods. Food Chem 2010; 122: 271-276.

34. Kanno T, Kuda T, An C, et al. Radical scavenging capacities of saba-narezushi, Japanese fermented chub mackerel, and its *lactic acid bacteria*. *Lebensm Wiss Technol* 2012; 47: 25-30.
35. Nyanzi R, Shuping DS, Jooste PJ, et al. Antibacterial and antioxidant activity of extracts from selected probiotic bacteria. J Food Res 2015; 4: 122-132.
36. Liu L, Qu X, Xia Q, et al. Effect of *Lactobacillus rhamnosus* on the antioxidant activity of Cheddar cheese during ripening and under simulated gastrointestinal digestion. Lwt 2018; 95: 99-106.
37. Chooruk A, Piwat S, Teanpaisan R. Antioxidant activity of various oral Lactobacillus strains. J Appl Microbiol 2017; 123: 271-279.
38. Amaretti A, Di Nunzio M, Pompei A, et al. Antioxidant properties of potentially *probiotic bacteria*: in vitro and in vivo activities. Appl Microbiol Biotechnol 2013; 97: 809-817.
39. Sun J, Hu XL, Le GW, et al. Lactobacilli prevent hydroxy radical production and inhibit *Escherichia coli* and *Enterococcus* growth in system mimicking colon fermentation. Lett Appl Microbiol 2010; 50: 264-269.
40. Ahmadi-Motamayel F, Goodarzi MT, Hendi, et al. Total antioxidant capacity of saliva and dental caries. Med Oral Patol Oral Cir Bucal 2013; 18: 553-556.
41. Lin MY, Chang FJ. Antioxidative effect of intestinal *bacteria Bifidobacterium longum ATCC 15708* and *Lactobacillus acidophilus ATCC 4356*. Dig Dis Sci 2000; 45: 1617-1622.
42. Songisepp E, Kullisaar T, Hütt P, et al. New probiotic cheese with antioxidative and antimicrobial activity. Int J Dairy Sci 2004; 87: 2017-2023.
43. Sun YP, Chou CC, Yu RC. Antioxidant activity of lactic-fermented Chinese cabbage. Food Chem 2009; 115: 912-917.
44. Lee NK, Han KJ, Son SH, et al. Multifunctional effect of probiotic *Lactococcus lactis KC24* isolated from kimchi. Lebensm Wiss Technol 2015; 64: 1036-1041.
45. Madhu AN, Amrutha N, Prapulla SG. Characterization and antioxidant property of probiotic and synbiotic yogurts. Probiotics Antimicrob 2012; 4: 90-97.
46. Guo YJ, Deng GF, Xu XR, et al. Antioxidant capacities, phenolic compounds and polysaccharide contents of 49 edible macro-fungi. J Function Foods 2012; 3: 1195–1205.
47. Ou C, Ko J, Lin M. Antioxidative effects of intracellular extracts of yogurt bacteria on lipid peroxidation and intestine 407 cells. J Food Drug Anal 2006; 14: 304-310.
48. Annuk H, Shchepetova J, Kullisaar T, et al. Characterization of intestinal *lactobacilli* as putative probiotic candidates. J Appl Microbiol 2003; 94: 403-412.
49. Balakrishnan G, Agrawal R. Antioxidant activity and fatty acid profile of fermented milk prepared by *Pediococcus pentosaceus*. J Food Sci Technol 2014; 51: 4138-4142.
50. Kullisaar T, Songisepp E, Mikelsaar M, et al. Antioxidative probiotic fermented goats' milk decreases oxidative stress-mediated atherogenicity in human subjects. Br J Nutr 2003; 90: 449-456.
51. Kudoh Y, Matsuda S, Igoshi K, et al. Antioxidative peptide from milk fermented with *Lactobacillus*

delbrueckii subsp. bulgaricus IFO13953. J Jpn Soc Food Sci 2001; 48: 44-50.

52. HernándezLedesma B, Miralles B, Amigo L, et al. Identification of antioxidant and ACE-inhibitory peptides in fermented milk. J Sc Food Agric. 2005; 85: 1041-1048.
53. Nishino T, Shibahara-Sone H, Kikuchi-Hayakawa H, et al. Transit of radical scavenging activity of milk products prepared by Maillard reaction and *Lactobacillus casei* strain Shirota fermentation through the hamster intestine. Int J Dairy Sci 2000; 83: 915-922.
54. Saiga A, Tanabe S, Nishimura T. Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. J Agr Food Chem 2003; 51: 3661-3667.
55. Sakanaka S, Tachibana Y, Ishihara N, et al. Antioxidant properties of casein calcium peptides and their effects on lipid oxidation in beef homogenates. J Agr Food Chem 2005; 53: 464-468.
56. Abadía-García L, Cardador A, Martín del Campo ST, et al. Influence of probiotic strains added to cottage cheese on generation of potentially antioxidant peptides, anti-listerial activity, and survival of probiotic microorganisms. Int Dairy J 2013; 33: 191-197.
57. Cumby N, Zhong Y, Naczk M, et al. Antioxidant activity and water-holding capacity of canola protein hydrolysates. Food Chem 2008; 109: 144-148.
58. Timón ML, Parra V, Otte J, et al. Identification of radical scavenging peptides (<3 kDa) from Burgos-type cheese. Lebensm Wiss Technol 2014; 57: 359-365.
59. Suetsuna K, Ukeda H, Ochi H. Isolation and characterization of free radical scavenging activities peptides derived from casein. J Nutr Biochem 2000; 11: 128-131.
60. Ou C, Lu T, Tsai J, et al. Antioxidative effect of lactic acid bacteria: intact cells vs. intracellular extracts. J Food Drug Anal 2009; 17: 209-216.
61. Osuntoki A, Korie I. Antioxidant activity of whey from milk fermented with *Lactobacillus* species isolated from Nigerian fermented foods. Food Sci Biotechnol 2010; 48: 505-511.
62. Lee J, Hwang KT, Chung MY, et al. Resistance of *Lactobacillus casei KCTC* 3260 to reactive oxygen species (ROS): role for a metal ion chelating effect. J Food Sci 2005; 70: 388-391.
63. Mishra V, Shah C, Mokashe N, et al. Probiotics as potential antioxidants: a systematic review. J Agric Food Chem 2015; 63: 3615-3626.
64. Archibald FS, Fridovich I. Manganese and defenses against oxygen toxicity in Lactobacillus plantarum. J Bacteriol 1981; 145: 442-451.
65. Farvin KS, Baron CP, Nielsen NS, et al. Antioxidant activity of yoghurt peptides: Part 1-in vitro assays and evaluation in ω-3 enriched milk. Food Chem 2010; 123: 1081-1089.
66. Lutgendorff F, Nijmeijer RM, Sandström PA, et al. Probiotics prevent intestinal barrier dysfunction in acute pancreatitis in rats via induction of ileal mucosal glutathione biosynthesis. PloS one 2009; 4: e4512.
67. Peran L, Camuesco D, Comalada M, et al. *Lactobacillus fermentum*, a probiotic capable to release glutathione, prevents colonic inflammation in the TNBS model of rat colitis. Int J Colorectal Dis 2006; 21: 737-746.
68. Grompone G, Martorell P, Llopis S, et al. Anti-inflammatory *Lactobacillus rhamnosus* CNCM I-3690 strain protects against oxidative stress and

increases lifespan in Caenorhabditis elegans. PloS one 2012; 7: e52493.

69. Kaizu H, Sasaki M, Nakajima H, et al. Effect of antioxidative *lactic acid bacteria* on rats fed a diet deficient in vitamin E. Int J Dairy Sci 1993; 76: 2493-99.
70. Afify AEMM, Romeilah RM, Sultan SI, et al. Antioxidant activity and biological evaluations of probiotic bacteria strains. Int J Acad Res 2012; 4: 131-139.
71. Lutgendorff F, Trulsson LM, Van Minnen L, et al. Probiotics enhance pancreatic glutathione biosynthesis and reduce oxidative stress in experimental acute pancreatitis. Americ J Physiol Gastrointest Liver Physiol 2008; 295: 1111-21.
72. Gupta A, Mann B, Kumar R, et al. Antioxidant activity of Cheddar cheeses at different stages of ripening. Int J Dairy Technol 2009; 62: 339-347.
73. Pritchard SR, Phillips M, Kailasapathy K. Identification of bioactive peptides in commercial Cheddar cheese. Int Food Res J 2010; 43:1545-1548.
74. Li B, Chen F, Wang X, et al. Isolation and identification of antioxidative peptides from porcine collagen hydrolysate by consecutive chromatography and electrospray ionization-mass spectrometry. Food Chem 2007; 102: 1135-1143.
75. Xie Z, Huang J, Xu X, et al. Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. Food Chem 2008; 111: 370-376.
76. Sadeghi N, Behzad M, Jannat B, et al. Total phenolic compounds content and antioxidant activity in packed and bulk milk in different regions of Tehran, Iran. J Food Safe & Hyg 2018; 4: 8-12.
77. Jafari M, Khaniki GJ, Roshanzamir M, et al. Antioxidant activity of raw milk and dairy products commonly consumed in Fars province, Iran. J Food Safe & Hyg 2017; 3: 21-26.
78. Peivasteh-Roudsari L, Pirhadi M, Karami H, et al. Probiotics and food safety: an evidence-based review. J Food Safe & Hyg 2019; 5: 1-9.