SCIENCE AND INNOVATION
INTERNATIONAL SCIENTIFIC JOURNAL VOLUME 3 ISSUE 5 MAY 2024
ISSN: 2181-3337 | SCIENTISTS.UZ

ANTIRADICAL ACTIVITY OF LOCAL STRAINS OF LACTOBACILLI

[1]Xazratkulova M.I., [2]Elova N.A., [3]Achilova J.A.
[1,2]Institute of Microbiology of the Academy of Science of the RUz
[3]Tashkent Pharmaceutical Institute
https://doi.org/10.5281/zenodo.11394029

Abstract*. The antiradical properties of local Lactobacillus strains were investigated using the free radical inhibition method of 1,1-diphenyl-2-picrylhydrazyl (DPPH). It was shown that both culture fluid and intact cells of the investigated caused a decrease in the level of DFPH radicals. The highest antiradical activity (41.9%) was demonstrated by Lacticaseibacillus rhamnosus AK 2.2: the inhibitory ability of the supernatant, is - 69.28%, and in intact cells is - 25.6%.*

Keywords*: free radicals, oxidants, oxidative stress, lactobacilli, antiradical activity, intact cells.*

Introduction. The term 'reactive oxygen species' (ROS) refers mainly to free radicals derived from molecular oxygen and some other chemically active substances whose molecules are formed by the gradual reduction of molecular oxygen. In the human body, AFCs act as regulators and mediators to ensure the proper functioning of cells [1]. The role of AFCs in many biological processes depends on their concentration, and overproduction can easily cause damage to proteins, nucleic acids or lipids as a result of free radical reactions [2]. Consequently, protective antioxidant mechanisms are activated when AFCs are overproduced. Oxidative stress occurs when these mechanisms do not function properly or efficiently, resulting in an imbalance between antioxidant and oxidant levels, with oxidation dominating. This imbalance affects human health and can contribute to the development of chronic diseases or ageing [3].

Peripheral fat deposition in the abdominal cavity and around internal organs leads to the development of lipotoxicity, which is one of the main causes of oxidative stress [4]. Oxidative stress and pro-inflammatory processes are closely related. Increased levels of pro-inflammatory cytokines lead to activation of the NADPH oxidase complex. This complex is localised on the plasma membranes of cells and in some organelles; it is the main source of reactive oxygen species [5].

Probiotics are popular dietary supplements because they have a variety of beneficial effects: improvement of the gastrointestinal microbial environment, competitive exclusion of pathogens and stimulation of the host immune system. Probiotic strains have been reported to scavenge hydroxyl radicals and superoxide anions and to produce antioxidants [3].

Probiotics can act directly by neutralising oxidants through the expression of antioxidant enzymes (one of the best known of these enzymes is SOD), produce various metabolites with antioxidant activity such as butyrate, glutathione (GSH) and folate, inhibit intestinal pathogens and reduce postprandial lipids involved in oxidative damage [6, 7].

Aim of study: To study the antioxidant activity of local strains of lactobacilli and to select strains for inclusion in the composition of preparations effective for weight loss in obese individuals.

Materials and methods. Twenty strains of lactic acid bacteria, isolated by the generally accepted method of isolating pure cultures of lactic acid bacteria from different sources, were used in the study. The following samples were used as sources of isolation: sauerkraut, hard cheese, camel milk, above-ground part of celery and faces of healthy infants.

Culture fluids, i.e. intact cells dissolved in phosphate buffer from the cultures under investigation, were used as the objects of study. Prior to the experiments, the cultures were stored in a freezer at -80o C. The antioxidant activity of the samples was determined according to the Glavin-Glavin method. The antioxidant activity of the tested samples was determined according to Glavind's method of free radical inhibition by 1,1-diphenyl-2-picrylhydrazyl (DPPH) [8].

Sample preparation. Lactic acid bacteria cultures were obtained by 2-fold resuspension in MRS-broth and incubation for 24 h at 37°C. Cells were precipitated by centrifugation at 16,000 rpm for 20 min. The culture supernatant, culture fluid (CF), was used to measure antioxidant activity. The precipitate was washed twice in isotonic solution and resuspended in 0.2 M phosphate buffer. The cell suspension was used for the measurement of antioxidant activity.

Determination of antiradical activity. The antiradical activity of the tested samples was determined according to the method of Glavind [8] by radical inhibition of 1,1-diphenyl-2-picrylhydrazyl (DFPH, CalBiochem, Germany).

A 0.2 mM alcoholic solution of DFPH (1 mL) was added to 1 mL of the test sample and mixed thoroughly. The resulting mixture was left in the dark for 30 min. The optical density was measured on a spectrophotometer at a wavelength of 517 nm. The titer of intact cells was adjusted to 109 CFU/mL. The rest of the procedure was as described above. Pure vitamin C (1 mg vitamin C + 1 ml distilled water) was used as a control. The antiradical activity was calculated as a percentage according to the formula:

Antiradical activity (%) = [1- (A_{sample} - A_{blind}) / A_{blank}] x 100,

Where Blind= phosphate buffer solution;

Blank= phosphate buffer solution + DPPG [8].

Results: The results for the determination of antiradical activity are shown in Figure 1. We observed a decrease in the optical density of DPPH solution when both culture fluid and intact lactobacilli cells were added. The antiradical activity of the supernatants of the studied cultures ranged from 50.94 to 69.28, the activity of intact cells was from 20.6 to 46.68.

Among the 20 cultures studied, the highest antiradical activity was found in the culture of Lacticaseibacillus rhamnosus AK 2.2: the inhibition capacity of the supernatant was 69.28%, while that of intact cells was 25.6%.

The antiradical activity of culture supernatants of P. acidilactici species ranged from 52.62 to 64.24%. The antioxidant activity of Limosilactobacillus fermentum strains ranged from 51.49 to 53.0%. The antioxidant activity of *Lacticaseibacillus rhamnosus* strains ranged from 53.63 to 69.28%. The antioxidant activity of Lactobacillus sp. strains ranged from 51.18 to 67.8%.

It is known that the most common forms of antioxidant defense in lactobacilli are related to the action of superoxide dismutase enzymes and high intracellular concentrations of Mn2+ ions, as well as peroxidase [9]. In addition to enzymes, various compounds may play a role in reducing the level of free radicals in lactobacilli cells. In particular, such properties have been shown for a low molecular weight fraction (< 10 kDa) obtained from the supernatant of lactobacilli cell lysate [10]. Many results also confirm the antiradical effect of components present in the supernatant of cell lysates.

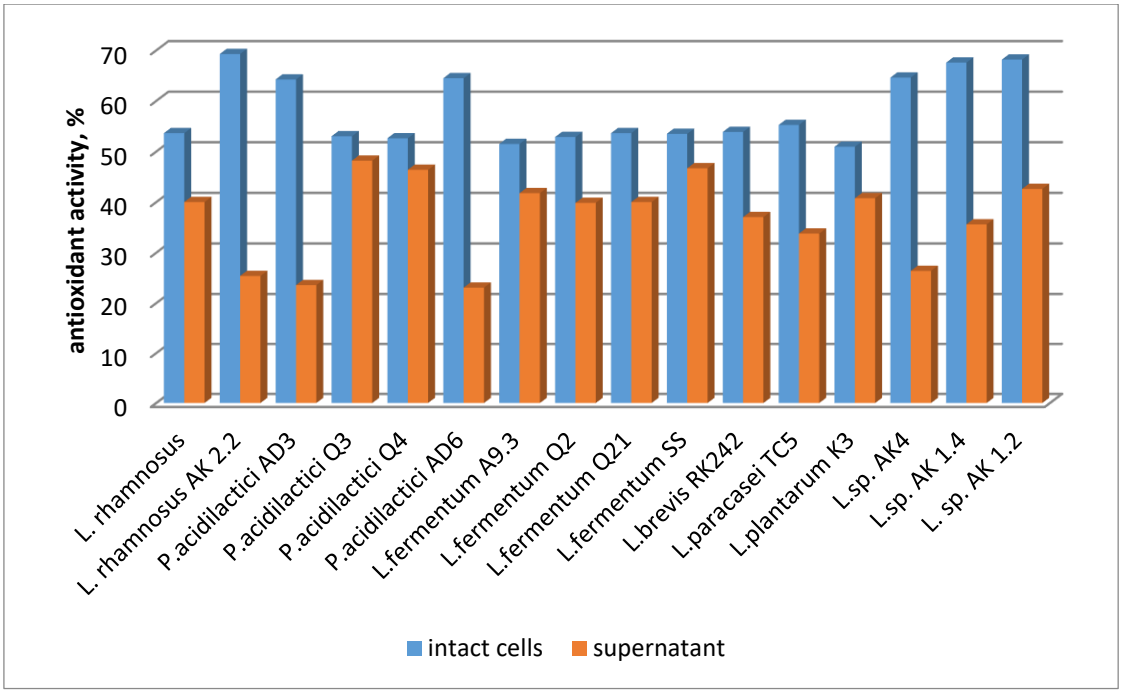

Figure 1. Antioxidant activity of strains

There is also evidence in the literature that lactobacilli exopolysaccharides have antioxidant properties [11].

Conclusions. Oxidative stress is a major cause of DNA damage. Based on our results, we can assume that the antiradical activity of local strains of lactic acid bacteria plays an important role in protecting DNA from damage in mammalian cells. Local strains of lactobacilli have a high free radical scavenging activity. Many of the strains studied can be considered as potential candidates for the development of functional foods effective in weight loss.

REFERENCES

1. Song P, Zou MH. Roles of reactive oxygen species in physiology and pathology. In: Atherosclerosis: Risks, Mechanisms, and Therapies//John Wiley & Sons 2015; 379-92.
2. Mateen S, Moin S, Khan AQ, Zafar A, Fatima N. Increased reactive oxygen species formation and oxidative stress in rheumatoid arthritis//PLoS One 2016; 11: e0152925.
3. Hoffmann A., Kleniewska P., Pawliczak R. Antioxidative activity of probiotics// Arch Med Sci 3, April / 2021. - PP. 792-804.
4. Agarwal A, Aziz N, Rizk B. Studies on Women's Health//HumanaPress, NewYork; 2013; pp 33–60. doi:https://doi.org/10.1007/978-1-62703-041-0.
5. Agarwal A, Aponte-Mellado A, Premkumar BJ, et al. The effects of oxidative stress on female reproduction: a review//Reprod Biol Endocrinol. 2012;10(1):49. doi: 10.1186/1477-7827-10-49.
6. Kapila S, Kapila R, Reddi S, Sinha PR. Oral administration of probiotic Lactobacillus casei spp. casei ameliorates oxidative stress in rats// Int J Curr Microbiol App Sci 2014; 3: 670-84.
7. Mikelsaar M, Zilmer M. Lactobacillus fermentum ME-3 an antimicrobial and antioxidative probiotic//Microb Ecol Health Dis 2009; 21: 1-27.
8. J. Glavind, Acta Chem Scand.,13, 1635—1640 (1963).

9. Карамова Н. С., Хабибуллин Р. Э. Антирадикальные свойства *Lactobacillus acidophilus* n.v.Ep. 317/402 IN VITRO// Вестник Казанского технологического университета. 2013. С.127-129.
10. Yamamoto Y., In Report of NISR Research GRANT//Young Investigator Research Grant, 2009. P. 57-58.
11. Elova N.A., Kutliyeva G.Dj., Zakiryaeva S.I., Bekmukhamedova N.K. Optimization of cultivation conditions for increasing the production of exopolysaccharides of the Lactobacillus plantarum EB-2 strain//Nat. Volatiles & Essent. Oils, 8(5), 8689-8697, 2021 https://nveo.org/index.php/journal/article/view/2265