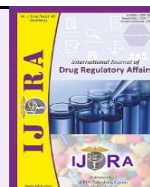


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Research Article

**The Role of Paraprobiotics in Breast Cancer Cell Line (MCF-7): In vitro study**Gulcin Alp Avci^{a,*}, Emre Avci^b^aDepartment of Basic Medical Sciences, Gulhane Faculty of Dentistry, University of Health Sciences, Ankara, Turkiye.^bDepartment of Biochemistry, Gulhane Faculty of Pharmacy, University of Health Sciences, Ankara, Turkiye.**Abstract**

Bioactive active metabolites seem promising in understanding the molecular pathology of cancer and especially in developing targeted therapies. In addition, the anticancer activity of some possible probiotic bacteria is emphasized in many literatures. Especially *Lactobacillus* strains and beneficial microbial activities are noteworthy. However, the use of live microorganisms in immunocompromised patients poses a risk. For this reason, our study aimed to evaluate the cytotoxic effect of inanimate/paraprobiotics obtained from probiotic microorganisms on the MCF-7 cell line, which is a breast cancer cell. *L. rhamnosus* ATCC 53103 and *L. reuteri* DSM 17938 strains and the MCF-7 cell line were used in our study. The cytotoxicity of paraprobiotics was determined by the MTT method. According to the obtained data, it was observed that paraprobiotics had a concentration and time dependent proliferative effect on healthy fibroblast cell lines and a cytotoxic effect dependent on the same parameters on MCF-7 cell lines. While the search for new pharmabiotic active molecules continues today, the identification of bioactive paraprobiotics will enable their use especially in individuals with immune system diseases and will allow the shelf life of biotic products to be longer.

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1. Introduction

Breast cancer is the most frequently diagnosed type of cancer in women Mondial and is almost the most common cause of cancer-related deaths in women. It has become an increasing health problem all over the world because it is the most widespread type of cancer in women and one of chiefly important causes of death. (1) Breast cancer diagnosis is most frequently followed worldwide with the TNM (primary tumor size: T; regional lymph node involvement: N; presence of distant metastasis: M) staging system defined by the American Joint Committee on Cancer (AJCC) to create a standardization in determining the extent of the disease and the risk of recurrence. (2)

The use of live bacteria in health applications can lead to risky situations in individuals with suppressed immune systems. In some cases, it can lead to the development of opportunistic pathogenicity. (3) These risks have recently led to new searches. Paraprobiotics are obtained because of exposure to factors such as mechanical damage to the cell membrane, breakage of DNA strands, inactivation of enzymes, and complete loss of cell viability in probiotics. (4) Inactivated microorganisms or processing of cell extracts that can be used as an alternative to live

microorganisms; It provides an important advantage such as providing a safer and easier use compared to probiotics, as it does not pose a risk of infection or inflammation in individuals with weak or suppressed immune systems. (5)

In our study aimed to evaluate the cytotoxic effect of inanimate/paraprobiotics obtained from probiotic microorganisms on the MCF-7 cell line, which is a breast cancer cell. *L. rhamnosus* ATCC 53103 and *L. reuteri* DSM 17938 strains and the MCF-7 cell line were used in our study.

1.1 Regulatory Consideration

Probiotics are defined, classified, and regulated differently across countries, which affects consumer protection and market access for manufacturers. This review examined the regulatory similarities and differences for probiotics in the APAC region, the U.S., and Europe to address these challenges.

Probiotics are categorized in the U.S., Europe, Japan, Thailand, Malaysia, Singapore, and Indonesia, with Malaysia, Singapore, and Indonesia also classifying them as "health supplements." Other terms like "functional food," "novel food," and "health functional food" are used

in various countries. Claims for probiotics also vary internationally. Countries such as China, the Philippines, and Singapore have pre-approved claims, while the U.S., South Korea, Australia & New Zealand, and India require scientific evidence before approval. Europe enforces the strictest rules and does not approve any probiotic health claims, whereas the U.S. and APAC regions are more

flexible. In Japan and Australia & New Zealand, the claim of “reducing disease risk” is permitted. (6)

Harmonizing these regulations could benefit consumers and manufacturers by ensuring consistent standards and facilitating market entry. This would help industry stakeholders navigate the complex regulatory landscape and aid consumers in understanding probiotic claims.

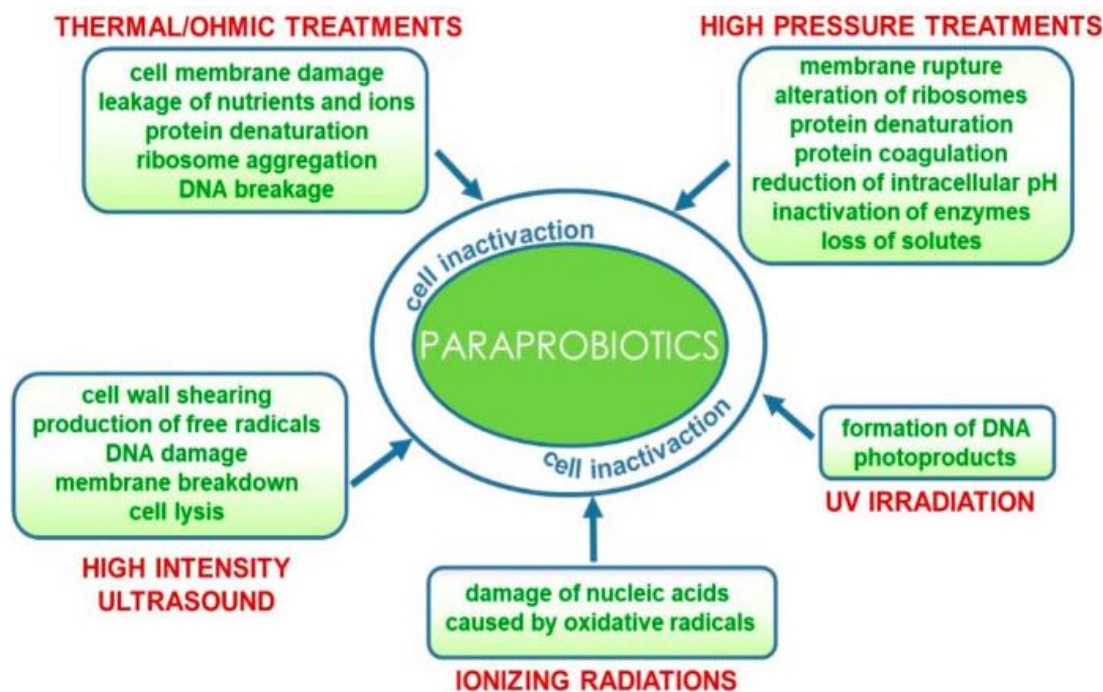


Figure 1. Technological processes for the production of paraprobiotics and their effects on bacterial cells. (7)

2. Material and Methods

Paraprobiotics were obtained from *L. rhamnosus* ATCC 53103 and *L. reuteri* DSM 17938 strains in our study. Bacterial strains were grown in MRS broth at 37°C for 18-24 hours and then centrifuged at 4200 rpm for 10 minutes at 4°C to obtain pellets and washed three times with saline solution (0.85% NaCl) and suspended in distilled water. Then, the bacteria were killed by heat treatment at 80°C for 20 minutes and centrifuged at 4200 rpm for 10 minutes at 4°C to obtain pellets and suspended in distilled water before being lyophilized and used.

In cytotoxicity studies, breast cancer cell line (MCF-7) and healthy fibroblast cell line (L929) was used. These cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, 1% penicillin-streptomycin for 24 h at 5% CO₂ and 37°C in 25 cm² cell culture flasks. The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT) method was used to determine cytotoxicity. (8) Paraprobiotics were added to confluent MCF-7 cell lines at different concentrations (500-250-125-62.5-31.25 mg/ml) and incubated. Cell viability was examined at 12 and 24 hours after incubation. EC₅₀ (half-maximal effective concentration) values were calculated.

Statistical Analysis

The data obtained were evaluated statistically through the SPSS program (IBM 22.0). The results were used graphically as mean values ± standard deviation.

3. Results

In our study, it was determined that paraprobiotics obtained from *L. rhamnosus* ATCC 53103 and *L. reuteri* DSM 17938 strains had a proliferative effect on the L929 cell line and a cytotoxic effect on the MCF-7 cell line with a correlation in parallel with the increasing concentration (Figure 2 & 3).

4. Discussion

The burden of cancer worldwide is increasing day by day. Breast cancer is the most common of these cancers. (9) In addition to the positive effects of probiotics on health, they are also known to prevent many types of cancer, suppress tumors and support treatment. (10) Goldin and Gorbach were among the first to report a reduction in the incidence of colorectal cancer (37% compared to controls) with a *Lactobacillus*-enriched feeding procedure.

The results of many in vitro studies highlight the beneficial properties of probiotics in regulating the proliferation and apoptosis of cancer cells in tissues such as the stomach, colon and breast. (11)

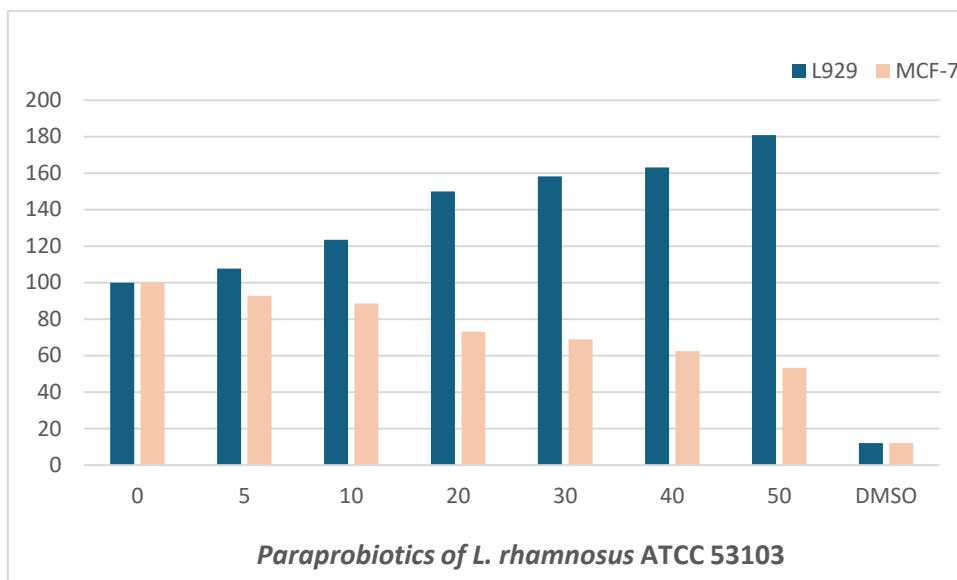


Figure 2. Cytotoxic effects of paraprobiotics from obtained *L. rhamnosus* ATCC 53103

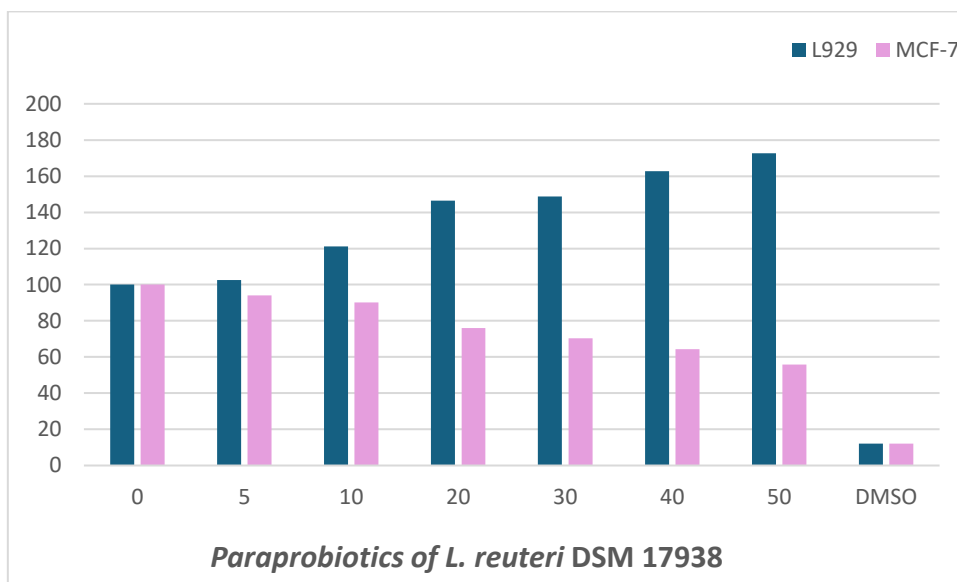


Figure 3. Cytotoxic effects of paraprobiotics from obtained *L. reuteri* DSM 17938

In many literatures examining probiotic-cancer interactions, it is reported that probiotics have positive effects on the changes in many metabolic activities such as strengthening the host's immune response, disrupting the structures of potential carcinogenic compounds, changes in intestinal flora, production of antimutagenic and anticarcinogenic compounds in the colon, and preventing the conversion of pre-carcinogens in intestinal microflora into carcinogens. (10-14) However, live bacteria applications pose a risk especially in individuals with suppressed immune systems. For this reason, the use of probiotics inactivated by various methods, just like the dead vaccines that have been used for years, has come to the fore. Data obtained from paraprobiotics also show that they are important bioactive supporters in many diseases.

5. Conclusion

According to the data obtained in our study, it was observed that paraprobiotics have a concentration and time-dependent proliferative effect on healthy fibroblast cell lines, and a cytotoxic effect on MCF-7 cell lines depending on the same parameters. While the search for new pharmacologically active molecules continues today, the identification of bioactive paraprobiotics will enable their use especially in individuals with immune system diseases and will provide a longer shelf life for biotic products.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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