International Journal of Innovative Technology and Interdisciplinary Sciences
https://ijitis.org/index.php/IJITIS
ISSN: 2613-7305
Volume 7, Issue 1
DOI: doi.org/10.15157/IJITIS.2024.7.1.15-23
Received: 04.01.2024 Revised: 07.02.2024 Accepted: 14.02.2024

Harnessing Probiotics: A Promising Avenue for Inhibiting Breast Cancer Cell Growth

Ambrozia Itellari*, Stacey L Raimondi

Department of Biology, Elmhurst University, USA
***aitel8751@365.elmhurst.edu, raimondis@elmhurst.edu**

Abstract

The prospective search for innovative treatments for breast cancer is underway, with vast research initiatives aimed at understanding factors that promote the development of breast cancer cells. Underlying factors that breast cancer cells have been determined to rely on are genetic factors, environmental variables, and the strength of the body's immune response. The resilience of the body's immune system is profoundly influenced by the diversity of a healthy gut microbiome. Probiotics, when utilized as supplements, confer microbial benefits by establishing microflora diversity. An abundance of probiotic bacteria within the body can enhance the production of anti-inflammatory cytokines and regulate hormones such as estrogen, which are relevant to combating breast cancer. The aim of the following research was to investigate the impacts of probiotics on breast cancer cell growth. The following research tested the hypothesis that an aggressive MDA-MB-231 and non-aggressive MCF-7 breast cancer cell line will experience inhibited growth in the presence of probiotics. It was determined that both breast cancer cell lines experienced a decrease in proliferation at the higher probiotic concentrations, with MCF-7 cells being the most negatively impacted by the highest probiotic concentration examined.

Keywords*:* Breast Cancer, Probiotics, Cell Proliferation, Inhibit, Microbiome, Estrobolome, Microflora, MCF-7, MDA-MB-231, Lactobacillus, Streptococcus

INTRODUCTION

Throughout the world, one in eight women is diagnosed with breast cancer annually, and one in three of these women will progress to develop metastatic breast cancer [1]. Underlying factors that breast cancer cells rely on are genetic factors, environmental variables, and the robustness of the individual body's immune response [2].

The strength of the immune response is intricately linked to the diversity of a flourishing gut microbiome [3]. Over the course of one's life, the gut microbiome experiences dynamic fluctuations in populational growth influenced by internal and external factors such as age, race, diet, infections and illnesses, and drug usage [4]. The human gastrointestinal tract harbors complex communities of microorganisms that assist with physiological processes including digestion, metabolism, preventing pathogen overgrowth, vitamin B and K production, and stimulation of cytokine production to initiate immune responses [5].

Maintaining the delicate balance of innate and adaptive immunity hinges significantly on the symbiotic relationship with gastrointestinal tract microorganisms. An imbalance in the microbiome renders individuals more susceptible to chronic diseases such as breast cancer. [7]. In fact, epidemiological studies exhibit a concerning correlation between patients overusing antibiotics and an increase in breast cancer cell development [8], indicating that a deficiency in beneficial microorganisms may play a role in promoting carcinogenesis.

The key role of beneficial microorganisms extends to the regulation and reabsorption of systemic estrogens [9]. Within the estrobolome, a collection of bacteria modulates hormones such as estrogen [5, 6].

The estrobolome's regulation of estrogen levels is executed through the management of microbial β-glucuronidase secretion. β-glucuronidase secretion synthesizes estrogen to its active form and makes estrogen accessible for tissue absorbance. However, when there is an overabundance of harmful bacteria present, it triggers excess β-glucuronidase secretion, leading to abnormal estrogen levels, a prevalent phenomenon in breast cancer patients [10,11]. Therefore, unregulated estrogen levels, induced by elevated β-glucuronidase activity, have been associated with the heightened susceptibility of patients to developing malignant hormone-driven breast cancer [12].

Recognizing the significance of maintaining a diverse and healthy microbiome has fuelled the rising popularity of probiotics. These live beneficial bacteria and yeast provide positive health benefits through consistent consumption and may promote beneficial immunomodulatory effects [13]. Probiotics increase the production of anti-inflammatory cytokines which are relevant to anticancer activity due to hindering carcinogenesis and eliminating early-stage cancer cells [14]. Particularly, it has been documented that probiotic strains like *L. acidophilus* (a component of Kefir microflora) effect the immune system by producing IL4 and IL10 anti-inflammatory cytokines, which have contributed to anticancer activity in breast tumors [15].

Current prevention methods and treatments for combating breast cancer are still underway, with most research focusing on innovative chemotherapy treatments for patients who have been diagnosed with breast cancer. Although chemotherapy is a viable method for treatment, noteworthy research studies have identified that the concurrent consumption of probiotics by patients undergoing chemotherapy leads to reductions in chemotherapy after-effects [16]. And thus, an intriguing avenue emerges to explore alternatives aimed at curbing the proliferation of breast cancer cells through the use of probiotics. Therefore, this study endeavors to test the hypothesis that the addition of probiotics to breast cancer cell lines will lead to a reduction in cell proliferation overall.

METHODS

The poorly invasive/metastatic MCF-7 and highly invasive/metastatic MDA-MB-231 breast cancer cell lines were procured from the American Type Culture Collection (ATCC), located in Manassas, Virginia, ensuring the reliability and authenticity of the cellular models used in the study. These cell lines were cultured in RPMI 1640 media and maintained under standard conditions of 37 degrees Celsius in 5% CO2. To investigate the potential impact of a probiotic cocktail on cell proliferation, a systematic experimental approach was employed. Specifically, $1x10^5$ cells from each cell line were seeded onto individual wells of a 6-well petri dish. The probiotic cocktail, sourced from Inner-Eco, Commerce City, Colorado, comprised strains of *Lactococcus lactis subsp, Leuconostoc subsp, Streptococcus thermophilus, Lactobacillus subsp,* and *Kefir grains microflora*. Different concentrations of the probiotic cocktail 0 µL, 12.5 µL, 25 µL, 50 µL, 100 µL, or 200 µL were incorporated into the designated wells to assess potential dose-dependent effects. After a one-week incubation period, the population growth of each breast cancer cell line was quantified using a hemocytometer. To ensure robustness and reliability, all experiments were conducted three times, and the results were subjected to rigorous statistical analysis. Data analysis involved calculating averages

and the populational standard deviations *SD* shown in equation 1, providing a quantitative measure of the observed trends.

$$SD = \sqrt{\frac{\sum_{i=1}^{N}(x_i - x_m)^2}{N}} \quad (1)$$

Where x_i is an individual measured value, x_m is the arithmetic mean value and N is the complete set of measurements.

Statistical significance was determined using a one-way analysis of variance (ANOVA) followed by the Tukey Honestly Significant Difference (HSD) post hoc test. Figure 1 depicts the schematic view of our measurement's setup.

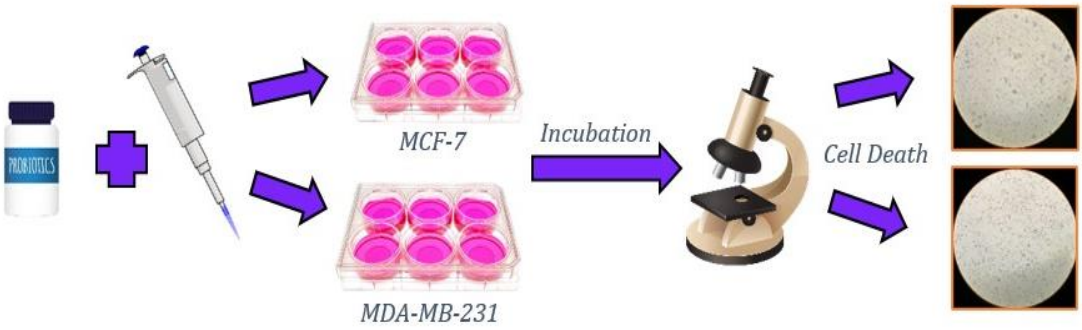
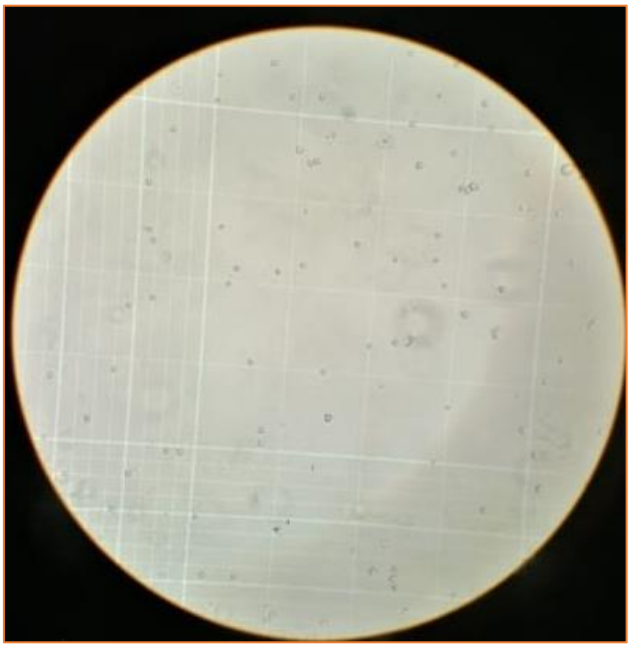


Figure 1. Schematic view of the measurement's setup

Furthermore, varying probiotic concentrations were inputted in 6-well-petri dishes, then incubated for a week. After cell death was observed, then the petri dishes underwent a washing procedure to count the remaining cancer cells. Figure 2 depicts the photographs of MCF-7 non-aggressive and MDA-MB-231 aggressive cancer cells.

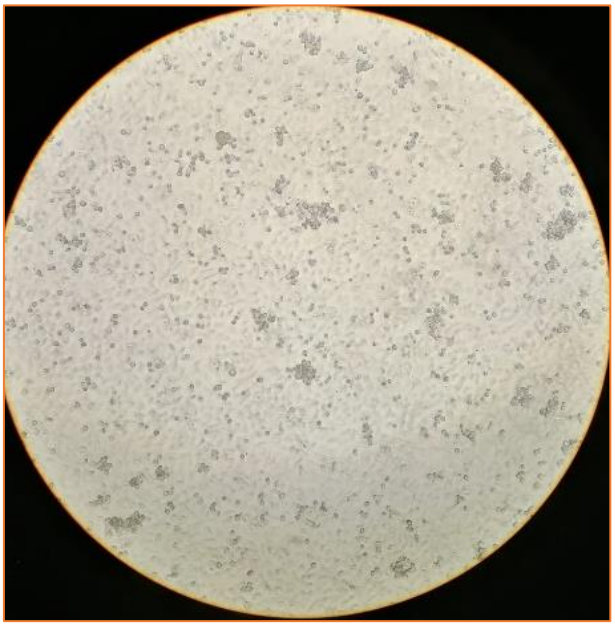
(a)



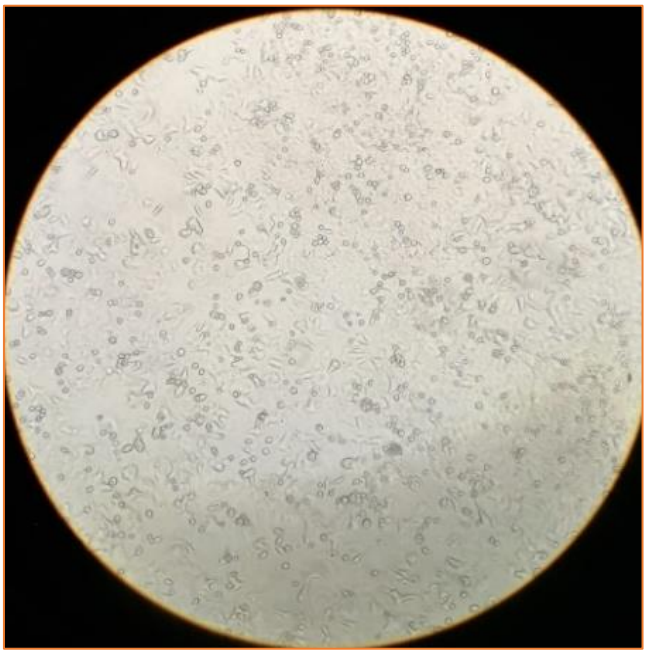
(b)

Figure 2. Photographs taken of the MCF-7 non-aggressive cancer cells (a) and the MDA-MB-231 aggressive cancer cells (b) under a microscope from the control.

Differential characteristics are observed when comparing both cancer cell lines side by side. In Figure 3, photographs of the aggressive and non-aggressive cancer cell lines under a microscope from the 200 μL probiotic dosing are shown.



(a)



(b)

Figure 3. Photographs taken of the MCF-7 (a) and MDA-MB-231 (b) cancer cell lines under a microscope from the 200 µL probiotic dosing.

The irregular-shaped ovals exhibit cancer cell death, and the firmly circular cells are the live remaining cancer cells. After these photos were taken, a washing procedure was conducted to remove the dead cells so that the live remaining cancer cells could be counted.

RESULTS

In order to determine the effects of probiotics on breast cancer cell proliferation, MCF-7 and MDA-MB-231 were treated with designated probiotic cocktail concentrations, and the growth rates of these cells were recorded (see Tables 1 and 2).

Table 1. Average cell proliferation of MCF-7 cells in varying concentrations of probiotics

	0 µL	**12.5 µL**	**25 µL**	**50 µL**	**100 µL**	**200 µL**
Trial 1	39.50	45.00	24.25	24.75	32.00	7.25
Trial 2	34.71	38.50	31.50	27.71	21.29	13.41
Trial 3	69.50	26.00	35.75	40.25	30.75	11.50
Average	47.90	36.50	30.50	30.90	28.01	10.72
Std Dev	18.86	9.66	5.81	8.23	5.86	3.16

As probiotic treatment concentration increased, the average number of cells in the 6-well petri dishes experienced a decline across both cancer cell lines throughout the week. The most noticeable changes are observed when comparing the control to the 200 µL probiotic treatments. Particularly in Figure 3, when conducting the Tukey test to compare the cancer cell treatments, there was a significant difference between the control group and 200 µL in

the MCF-7 cells with a P-value of p = 0.00649. When analyzing the overall concentrations across three trials, the MCF-7 data had a P-value of 0.01583 and the MDA-MB-231 cells had a P-value of 0.04573. Therefore, the combined data show that there is a statistically significant decline in cancer cell proliferation when treated with probiotics.

Table 2. Average cell proliferation of MDA-MB-231 cells in varying concentrations of probiotics

	0 µL	12.5 µL	25 µL	50 µL	100 µL	200 µL
Trial 1	31.75	28.50	16.00	24.50	21.00	8.75
Trial 2	33.83	23.91	21.87	8.17	6.71	2.04
Trial 3	26.00	35.25	41.25	30.75	16.00	12.25
Average	30.53	29.22	26.38	21.14	14.57	8.01
Std Dev	4.06	5.70	13.21	11.66	7.25	4.64

The average number of results of MCF-7 and MDA-MB-231 cells after 7 days with the designated probiotic treatment concentrations is shown in Figure 4.

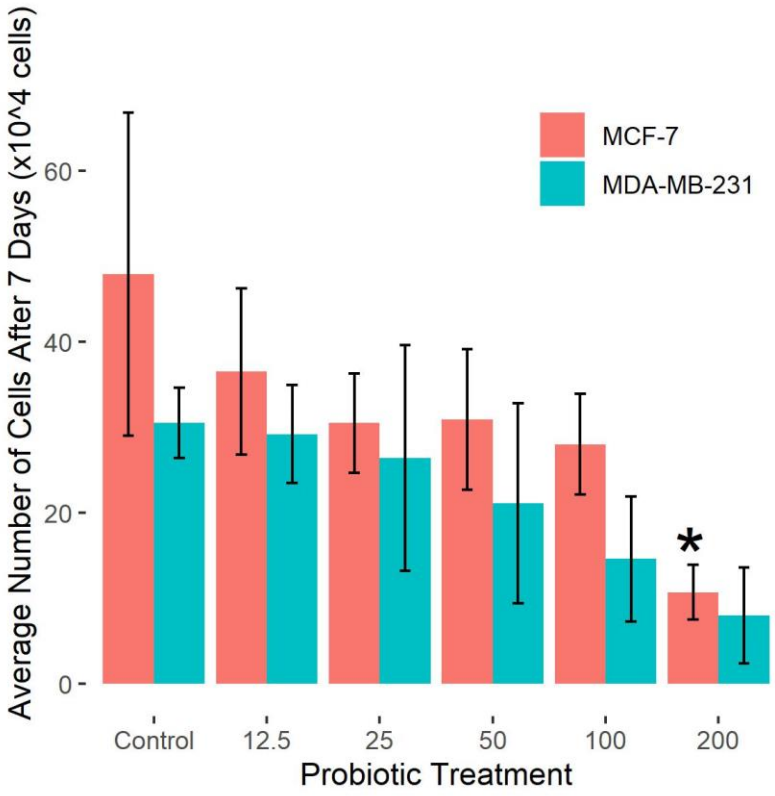


Figure 4. The average number of MCF-7 and MDA-MB-231 cells after 7 days with the designated probiotic treatment concentrations.

CONCLUSION

MCF-7 and MDA-MB-231 breast cancer cell lines have played pivotal roles in research, each offering unique characteristics that prove beneficial in specific aspects of breast cancer studies. MCF-7 cells have been notably profound in understanding the correlation between estrogen levels and breast cancer cell development, as well as being used in studies of estrogen receptor-positive breast cancer [17]. On the other hand, known for their aggressive and metastatic nature, MDA-MB-231 cancer cells are crucial in exploring breast cancer metastasis due to their highly invasive behavior [18].

Building upon previous studies involving these breast cancer cell lines, our research aimed to investigate the potential impact of a blend of beneficial probiotic strains on the proliferation of different breast cancer cells. As Figure 3 reveals, when the probiotic concentration increased, the average cell proliferation for both MCF-7 and MDA-MB-231 cells declined. Specifically, as indicated from Tables 1 and 2, there was an average of 47.90 x 104 MCF-7 and 30.53 x 104 MDA-MB-231 cells in the control groups; however, the average cell count declined through to the 200 µL probiotic concentration, in which there were 10.72 x 104 MCF-7 cells and 8.01 x 104 MDA-MB-231 cells. While the declining breast cancer cell proliferation was statistically significant in both cell lines, when conducting the Tukey test, it was identified that there was only pair-wise significance in the MCF-7 cells when comparing the control group to the 200 µL probiotic concentration. Although this was the case, the overall decline in cancer cell growth across the different probiotic concentrations shows a correlation between the effectiveness of probiotics in treating breast cancer cells.

Future trials are warranted to explore the additional pair-wise significance of probiotics impacting MDA-MB-231 cells. Presently, the data suggests that probiotics exhibit greater efficacy in treating MCF-7 cancer cells to reduce proliferation. Previous research emphasizing the role of the estrobolome may elucidate the more pronounced effect MCF-7 cells have when compared to MDA-MB-231 cells [6]. Given that MCF-7 cells are estrogen receptor-positive, this may explain why they are more susceptible to probiotic influence than MDA-MB-231 cells.

Despite the efficacy of probiotics against cancer cell lines, the challenge lies in determining the appropriate dose of probiotics to be administered to patients. Although probiotics are potentially effective against cancer cell lines, the dose needed would be difficult to recreate safely with the current state of technological advancements. Achieving these benefits would necessitate the daily consumption of approximately one liter of the probiotic cocktail, presenting a logistical challenge. Nevertheless, recognizing the benefits of supplemental probiotics in reducing cancer cell proliferation provides valuable insights for breast cancer preventative measures and treatments in the future.

ACKNOWLEDGMENT

I extend my heartfelt gratitude to the Elmhurst University Biology Department and Dr. Stacey L. Raimondi for their unwavering encouragement, steadfast support, and invaluable guidance throughout the entirety of this research endeavor.

Additionally, I express my sincere appreciation to Elmhurst University for providing me access to the cancer cell lines and laboratory facilities, which played a crucial role in making this research possible.

CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

REFERENCES

[1] Centres for Disease Control and Prevention, Breast Cancer in Young Women. Available at https://www.cdc.gov/cancer/breast/young_women/bringyourbrave/breast_cancer_young_women/index.htm Accessed on 1st March 2023.

[2] Celebioglu, H. Probiotic bacteria grown with chestnut honey enhance in vitro cytotoxicity on breast and colon cancer cells. *Archives of Biological Sciences*, 2020; 72(3); 329-338.

[3] Belkaid Y., Hand T. Role of the microbiota in immunity and inflammation. *Cell*, 2014; 157(1); 121-141.

[4] Durack J., Lynch S. The gut microbiome: Relationships with disease and opportunities for therapy. *J. Exp. Med.*, 2019; 216(1); 20-40.

[5] Mendoza L. Potential effect of probiotics in the treatment of breast cancer. *Oncol. Rev*., 2019; 13(2); 422.

[6] Kwa M, Plottel C.S., Blaser M.J., Adams S. The Intestinal Microbiome and Estrogen Receptor-Positive Female Breast Cancer. *J. Natl. Cancer Inst.*, 2016; 108(8); 1-10.

[7] Wu H.J., Wu E. The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes*., 2012; 3(1); 4-14.

[8] Xuan C., Shamonki J.M., Chung A., Dinome M.L., Chung M., Sieling P.A., Lee D.J. Microbial dysbiosis is associated with human breast cancer. *PLoS One*., 2014; 9(1); 0083744.

[9] Gloux K., Berteau O., El Oumami H., Béguet F., Leclerc M., Doré J. A metagenomic β-glucuronidase uncovers a core adaptive function of the human intestinal microbiome. *Proc. Natl. Acad. Sci. USA*., 2011; 108(1); 4539-4546.

[10] Ervin S.M., Li H., Lim L., Roberts L.R., Liang X., Mani S., Redinbo M.R. Gut microbial β-glucuronidases reactivate estrogens as components of the estrobolome that reactivate estrogens. *J. Biol. Chem*., 2019; 294(49); 18586-18599.

[11] Sui Y., Wu J., Chen J. The Role of Gut Microbial β-Glucuronidase in Estrogen Reactivation and Breast Cancer. *Front Cell Dev Biol*., 2021; 9; 631552.

[12] Yue W., Wang J.P., Li Y., Fan P., Liu G., Zhang N., Conaway M., Wang H., Korach K.S., Bocchinfuso W., Santen R. Effects of estrogen on breast cancer development: Role of estrogen receptor independent mechanisms. *Int. J. Cancer*, 2010; 127(8); 1748-1757.

[13] Kechagia M., Basoulis D., Konstantopoulou S., Dimitriadi D., Gyftopoulou K., Skarmoutsou N., Fakiri E.M. Health benefits of probiotics: a review. *ISRN Nutr*., 2013; 481651.

[14] LeBlanc de M. de A., Matar C., Farnworth E., & Perdigón G. Study of immune cells involved in the antitumor effect of kefir in a murine breast cancer model. *Journal of Dairy Science*, 2007; 90(4); 1920-1928.

[15] Imani Fooladi A.A., Yazdi M.H., Pourmand M.R., Mirshafiey A., Hassan Z.M., Azizi T., Mahdavi M., Soltan Dallal M.M. Th1 Cytokine Production Induced by Lactobacillus acidophilus in BALB/c Mice Bearing Transplanted Breast Tumor. *Jundishapur J. Microbiol*., 2015; 8(4); 17354.

[16] Feng J., Gao M., Zhao C., Yang J., Gao H., Lu X., Ju R., Zhang X., Zhang Y. Oral Administration of Probiotics Reduces Chemotherapy-Induced Diarrhea and Oral Mucositis: A Systematic Review and Meta-Analysis. *Front Nutr*., 2022; 9; 823288.

[17] Vantangoli M.M., Madnick S.J., Huse S.M., Weston P., Boekelheide K. MCF-7 Human Breast Cancer Cells Form Differentiated Microtissues in Scaffold-Free Hydrogels. *PLoS One*, 2015; 10(8); 0135426.

[18] Hero T., Buhler H., Kouam P., Priesch-Grzeszowiak B., Lateit T., Adamietz I. The Triple-Negative Breast Cancer Cell Line MDA-MB 231 Is Specifically Inhibited by the Ionophore Salinomycin. Anticancer Research, *Anticancer Research*, 2019; 39(6); 2821-2827.