



Primary Evidence on the Potential of *Lactobacillus paracasei* in Treatment of Hepatocellular Carcinoma

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Abstract

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Introduction: Hepatocellular carcinoma (HCC) is the 6th human cancer with high morbidity and mortality rate. Owing to the recent finding of anticancer effects of probiotics in various types of human cancers, the current study was conducted to assay the potential of *Lactobacillus paracasei* as a main microbial ingredient of dairy products in inhibition of HCC cell growth.

Methods: HepG2 cell line was treated with different concentration of supernatant isolated from *Lactobacillus paracasei* strain TD3 and MRL/MRS broth mediums as a negative control. MTT assay was performed to assess cell viability, 24 hours following treatment.

Results: MTT results demonstrated a significant decrease in HepG2 cells viability, which was directly correlated with the concentration of bacterial supernatant.

Conclusion: Similar to the other human cancers, *Lactobacillus paracasei* can be considered as a potential anticancer probiotic in restriction of HCC cells growth. Further studies are needed to clarify the active components of *L. paracasei* supernatant and its potential as adjuvant therapy in HCC patients' treatment, as well.

Keywords: *Lactobacillus paracasei*, HepG2, Cell line, Treatment

Introduction:

Hepatocellular carcinoma (HCC) is the most frequent primary liver tumor which usually arises following cirrhosis with high mortality rate and it is considered as the 6th of human cancers.

Main risk factors of HCC are included chronic hepatitis B virus infection, fatty liver, dietary related factors such as carcinogenic mycotoxins as well as aflatoxin, alcohol, insulin resistance and food compounds with the potential to produce reactive oxygen species. Food-related

risk factor elements, as well as aflatoxin, can attenuate critical cancer network genes including *p53* and *BCL2* genes expression and thereby accelerates tumorigenesis evolution.¹⁻⁴ Owing to the more frequency in the younger population and the pivotal role of dietary habits on the risk of HCC, including the best supplementary food elements as well as probiotics in routine human diet have shown to be effective in the prevention of various diseases such as different types of human cancers.



Probiotics actually are viable micro-organisms which have been demonstrated to be effective in improving human body microbiome as well as the microbial flora of intestine and vagina. *Lactobacilli* as one of the major traditional probiotic classification were frequently used to be included in dairy products and it is considered as GRAS (Generally Recognized as Safe) organism, which can be incorporated in pharmaceutical products.⁵ The promising effects of various strains of *Lactobacillus* have been demonstrated on augmentation of immune system function, antimutagenic and antitumor effects and also decreasing in serum cholesterol and sugar levels which can indirectly reverse the carcinogenic process in all cancer patients especially cirrhotic ones.⁶⁻⁸

In this regard, we were aimed to investigate the effect of *Lactobacillus paracasei* strain TD3 supernatant on HepG2 cell line viability to primarily determine its anticancer effects on HCC.

Materials and Methods

Probiotic culture and preparation

Lactobacillus paracasei strain TD3 was subjected to be cultured in de Man Rogosa Sharpe (MRS) broth medium (pH=6.5, Merck, Germany) for 48 h. Optical density (OD) of bacterial culture was then determined using spectrophotometer to be optimized as 0.7-0.8 at 600 nm of UV wavelength which was determined by plate counting on MRS agar (equals to 10^9 CFU/ml bacterial number).

Bacterial cell cultures were centrifuged at $1100 \times g$ for 15 minutes at $4^\circ C$ to separate bacterial supernatant from cell pellets and their pH was adjusted to be neutralized (pH =7.2).

The separated bacterial supernatant was filtered using 0.2 μm membrane filter to be sterilized and free of any bacterial cells and debris before cell line treatment.

Cell culture

HepG2 cell line was purchased from Iranian Biological and Genetic Resources Center. It was cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with L-glutamine, 10% (vol/vol) fetal bovine Serum (FBS, Gibco, USA) and %1 antibiotic solution (10.0 U/ml penicillin G sodium, 10.0g/ml streptomycin sulfate (Gibco, USA). Harvested HCC cells were transferred to 96-well tissue culture plates at the density of 1×10^4 cells per well. Monolayers of HepG2 cells were incubated for 24 h to be confluent before treatment step. The supernatant of *L. paracasei* culture preparation (10^9 CFU/ml) was added to the monolayer of HepG2 cells with concentration of 5, 10, 20, 30 and 40 % (v/v) in triplicates and then was incubated in a humidified incubator (5% CO₂ at $37^\circ C$). Negative control wells were treated with serial dilutions of MRS and MRS broth adjusted with lactate (MRL, pH=4.35) mediums.

MTT ASSAY

To measure the cytotoxic effect of *Lactobacillus paracasei* TD3, 20 μl of MTT solution [5 mg/ml in phosphate-buffered saline (PBS)] was added to the cell culture medium following treatment of cells with bacterial supernatant after 24 h. The cell plates were incubated for 4 hours and then their solutions were replaced by 100 μl DMSO to solubilize formazan blue crystals. Absorbance was measured at 570 nm using ELISA Reader instrument (Biotek, Absorbance Microplate Reader, USA) after 15 minutes. The cell viability was calculated as the following formula:

Viability (percentage of control) = [(absorbance sample-absorbance blank)/(absorbance control-absorbance blank)] $\times 100$.





Results

Bacterial supernatant concentrations which were used in treatment were calculated in \log_{10} to be analyzed in GraphPad Prism (version 7.03). The equivalent calculated logs were 0.69, 1.00, 1.30, 1.47, 1.60 for 5, 10, 20, 30 and 40 % of HepG2 cells concentration, respectively

(Fig.1). MTT assay graph demonstrated that TD3 had a significant inhibitory effect on HepG2 cell growth in comparison with negative controls including cells treated with control MRL and MRS solutions. MRL control result was a confirmation of this fact that the acidity was not the main cause of HepG2 cell growth inhibition.

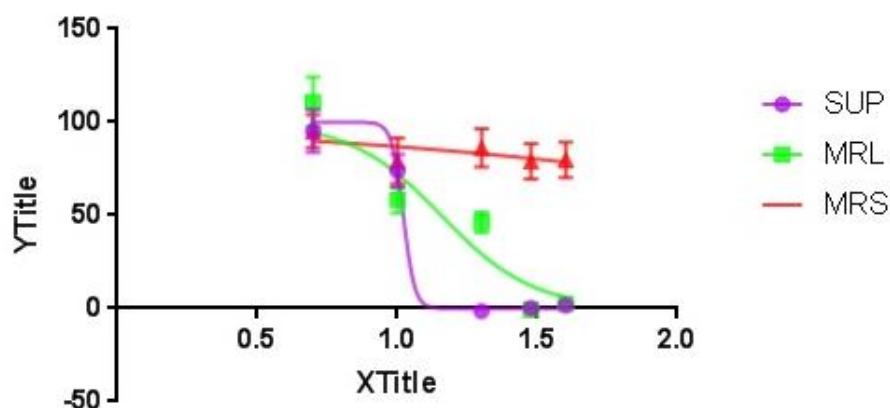


Figure 1: Inhibitory effect of *Lactobacillus paracasei* TD3 on HepG2 cell growth. Y-axis is indicating cell viability percentage (%) whereas X-axis is showing the \log_{10} of bacterial supernatant concentration; SUP: supernatant, MRS: man rogosa sharpe, MRL: MRS broth adjusted with lactate.

Discussion

Due to the possibility of including probiotics in dietary schedules and regimens and even as an ingredient of routine foods as well as various types of cakes and confectionaries, their nutritious values in prevention and control of different human diseases cannot be negligible. In this regard, various studies have investigated the effects of different strains of probiotics on cancer cells growth and toxicity to assess their potential as an anticancer supplement or maybe therapeutic drugs in adjuvant therapy. Herein, it was primarily shown that HCC cells toxicity has been increased in higher concentrations of *L. paracasei* TD3 treatment.

Intestinal microbiome could affect liver health and function through the liver-gut axis and transportation of lipopolysaccharides (LPS)

absorbed from pathogenic bacteria via the portal vein, which can be modulated by probiotics microbial flora.⁹ To the best of our knowledge, the literature on the treatment of HCC cell lines with probiotics are limited and their potential as either preventive or therapeutic food supplements is only its primary focus.¹⁰

The capability of cancer cell growth confinement by *Lactobacillus paracasei* alone or in combination with other chemotherapeutic drugs has been replicated in many studies performed on other cancer cell lines. Faghfoori *et al.* in the study on the treatment of *Lactobacillus* isolated from yogurt including *L. paracasei* on human colon cancer cell lines (HT-29 and caco-2) have demonstrated that cancer cells viability has been significantly



decreased compared to control cells. ¹¹ Chang *et al.* have shown the same results when treated with another colorectal cancer cells (CT26) with *L. paracasei* combined with 5-fluorouracil (5-FU). ¹² In another study that performed on erythroleukemia derived cancer cells (K562 cell line), significant suppression of cancer cells proliferation has been shown to be induced by *L. casei* and *paracasei* treatment. ¹³ Hu *et al.* also have demonstrated that *L. paracasei* caused cell cycle arrest and induced apoptosis in colorectal cancer cells through activation of reactive oxygen species (ROS) meanwhile decreasing in antioxidant enzymes as well as superoxide dismutase (SOD). ¹⁴ Duarte *et al.* also have shown promising inhibitory effects on human breast cancer cell lines (T47D and MDA-MB-231) induced by treatment with *L. paracasei*. ¹⁵

Given that most studies investigated the effects of *L. paracasei* on cancer cell inhibition have found hopeful results, the next steps will be determining active components of bacterial supernatant with anticancer characteristics. In this way, elucidating the involved signaling pathways and enrolled genes not only will describe the molecular mechanisms behind *L. paracasei* anticancer effects, but also may reveal novel active molecules in the carcinogenesis process with the potential of targeting. Further investigation in the aforementioned horizons is strongly warranted to be performed on HCC cells and animal model to precisely determine the anticancer potential of *L. paracasei* as an active bacterial constituent of dairy products.

Conflict of interest

The authors declare no conflicts of interest.

References

1. Qi, L.N., *et al.*, The p53 mutation spectrum in hepatocellular carcinoma from Guangxi, China: role of chronic hepatitis B virus infection and aflatoxin B1 exposure. *Liver Int* 35(3): 999-1009 (2015).
2. Imani Fooladi, A.A., *et al.*, Probiotic as a novel treatment strategy against liver disease. *Hepat Mon* 13(2): e7521 (2013).
3. Schlageter, M., *et al.*, Histopathology of hepatocellular carcinoma. *World J Gastroenterol* 20(43): 15955-64 (2014).
4. Waghray, A., *et al.*, Hepatocellular carcinoma complicating recurrent hepatitis C after liver transplantation. *Hepatology* 58(5): 1854-5 (2013).
5. Shoaib, A., W. Dachang, and Y. Xin, Determining the role of a probiotic in the restoration of intestinal microbial balance by molecular and cultural techniques. *Genet Mol Res* 14(1): 1526-37 (2015).
6. El Hage, R., E. Hernandez-Sanabria, and T. Van de Wiele, Emerging Trends in "Smart Probiotics": Functional Consideration for the Development of Novel Health and Industrial Applications. *Front Microbiol* 8: 1889 (2017).
7. Bermudez-Brito, M., *et al.*, *L. plantarum* WCFS1 enhances Treg frequencies by activating DCs even in absence of sampling of bacteria in the Peyer Patches. *Sci Rep* 8(1): 1785 (2018).
8. Iwasa, F., S. Sassa, and A. Kappas, Aminolaevulinate synthase in human HepG2 hepatoma cells. Repression by haemin and induction by chemicals. *Biochemical Journal* 262(3): 807-813 (1989).
9. Seki, E. and B. Schnabl, Role of innate immunity and the microbiota in liver





- fibrosis: crosstalk between the liver and gut. *J Physiol* 590(3): 447-58 (2012).
10. Wan, M.L.Y. and H. El-Nezami, Targeting gut microbiota in hepatocellular carcinoma: probiotics as a novel therapy. *Hepatobiliary Surg Nutr* 7(1): 11-20, (2018).
 11. Faghfoori, Z., *et al.*, Prophylactic effects of secretion metabolites of dairy *Lactobacilli* through downregulation of ErbB-2 and ErbB-3 genes on colon cancer cells. *Eur J Cancer Prev* (2017). doi: 10.1097/CEJ.0000000000000393.
 12. Chang, C.Y. and T.M. Pan, Anticancer and Antimigration Effects of a Combinatorial Treatment of 5-Fluorouracil and *Lactobacillus paracasei* subsp. *paracasei* NTU 101 Fermented Skim Milk Extracts on Colorectal Cancer Cells. *J Agric Food Chem* 66(22): 5549-5555 (2018).
 13. Nozari, S., *et al.*, The Study of Extracellular Protein Fractions of Probiotic Candidate Bacteria on Cancerous Cell Line. *Arch Iran Med* 19(11): 779-785 (2016).
 14. Hu, P., *et al.*, *Lactobacillus paracasei* subsp. *paracasei* M5L induces cell cycle arrest and calreticulin translocation via the generation of reactive oxygen species in HT-29 cell apoptosis. *Food Funct* 6(7): 2257-65 (2015).
 15. Duarte, C., *et al.*, Effects of biosurfactants on the viability and proliferation of human breast cancer cells. *AMB Express* 4: 40 (2014).

