

# ANTICANCER ACTIVITY OF PROBIOTICS – EXPERIMENTAL AND CLINICAL STUDIES

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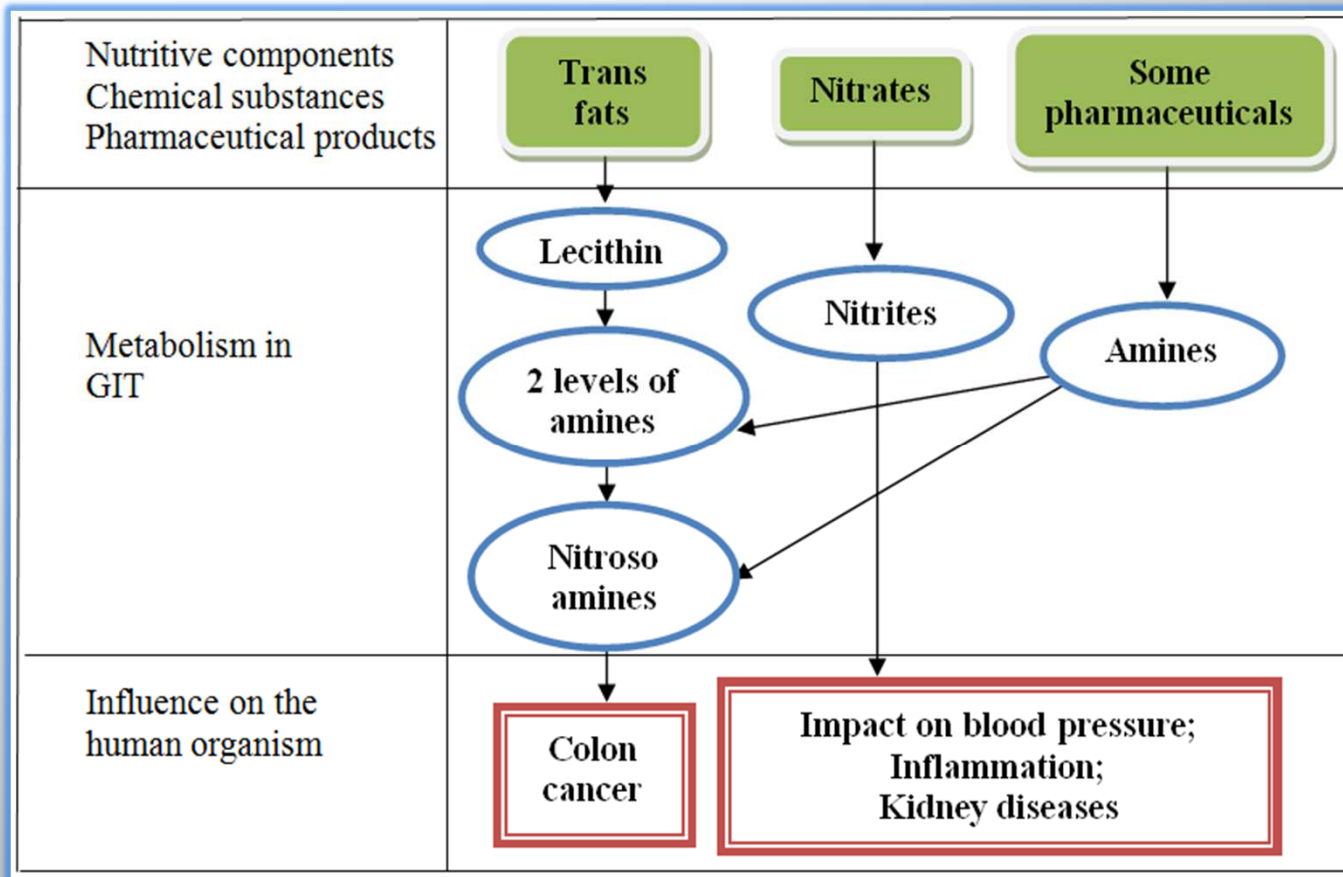
# Introduction

- **Probiotics according to WHO/FAO are live microorganisms which when consumed in adequate amounts as part of food confer a health benefit on the host.**
- **A man lives in coexistence with over 1500 types of microorganisms. They inhabit the whole organism – the mouth, skin, hair, vagina. Most of them are located in the colon - over 600 types of microorganisms weighing 1,5 - 2kg.**
- **According to the effect on the human organism the microorganisms are divided to pathogenic (150 – 180 types), beneficial like probiotics (15 – 20 types) and latent, which can be conditionally useful, conditionally harmful or not enough studied (1000 – 1200 types).**

# Introduction

- **The microorganisms that inhabit the human body form the internal microbial flora. It can be individual for every human being and common for people located in certain geographical area with similar lifestyle.**
- **The different microorganisms in that specific microbial ecosystems are usually in stable equilibrium in the healthy organism.**
- **Unhealthy diet, usage of antibiotics, pesticides, nitrates, toxins and other can lead to disruption to microbial balance, dysbacteriosis and different illnesses.**

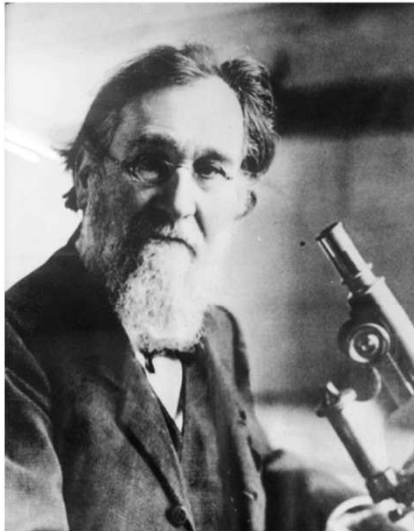
# Metabolism of some substances in GIT and diseases



**Modern researches show that diseases like GI infections, colitis, hepato-biliary diseases, allergies are caused by disbalance in the internal microbial flora.**

**The Probiotics maintain the internal microbial balance and regulate the functions of the gastrointestinal tract.**

# Probiotics - history



I. Mechnikov  
1845-1916

"Our aging  
is a disease  
that must be treated  
like any other  
disease."

A handwritten signature in cursive script, reading "Ilya Mechnikov". The signature is written in dark ink on a light-colored, textured background.

The foundations of the modern study of Probiotics is laid in the beginning of XX century by the Nobel Prize winner Ilya Mechnikov. He visit 28 countries and writes:

**"The longevity of the population in the Bulgarian lands is due to the daily consumption of yogurt and other products, containing lactic acid bacteria". At that time in Bulgaria there are 4 per thousand centenarians and low cancer morbidity rate.**

# Lactobacillus Bulgaricus - history

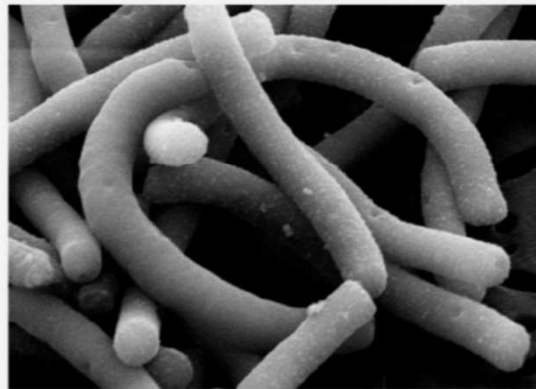
In 1905 in Geneva, the Bulgarian student St. Grigorov isolated the specific Bulgarian Lactobacillus from yogurt, brought from Bulgaria.

In 1914 the bacteria gets its name Lactobacillus Bulgaricus.



**Stamen Grigorov**  
Bulgarian Biologist  
and Physician  
1878-1945

Lactobacillus delbrueckii  
subsp. bulgaricus



Lactobacillus Bulgaricus is the only probiotic microorganism bearing the name of a certain geographical territory and nation. In other geographic regions of the World it mutates and stops its reproduction. During fermentation only L. Bulgaricus produces D(-) lactic acid in contrast to all other lactic acid bacteria.

# Antitumor effects of *Lactobacillus Bulgaricus* – researches

For over 100 years are carried out numerous studies on the antitumor & ray protective effects of different strains of *Lactobacillus Bulgaricus*.

- In 1956 I. Bogdanov proves that strain *L. Bulgaricus* LB51 inhibits the growth and spread of cancerous cells in mice. When intraperitoneal injected in mice with Crocker sarcoma (S180), the strain *L. Bulgaricus* LB51 heal 30% of the animals completely. Similar results are gathered by trials with Ehrlich tumor. In the cured animals by reinjection with Crocker or Ehrlich tumor, the cancer can not develop. Bogdanov speaks of anticancer immunity.
- Multiple studies carried out on male Wistar rats in the period 1985 – 2010 by different Bulgarian scientists (N. Aleksandrov, B Gyosheva, M. Georgieva) show good ray protective effect of strain *L. Bulgaricus* LBL4 and LB144. The strains increase the resistance of the bloodforming system to nonlethal gamma irradiation, which can be explained by strengthening of the immune activity of the body and suppressing lipid peroxidation.

# Antitumor effects of *Lactobacillus Bulgaricus* – researches

➤ An experimental study carried out in 2005 by R. Enikova shows antitumor effects of strains *L. Bulgaricus* Lb4 and Lb5 on chemically induced tumorigenesis in male white Wistar rats. The strains *L. Bulgaricus* Lb4 and Lb5 suppress and delay the Colon tumorigenesis.



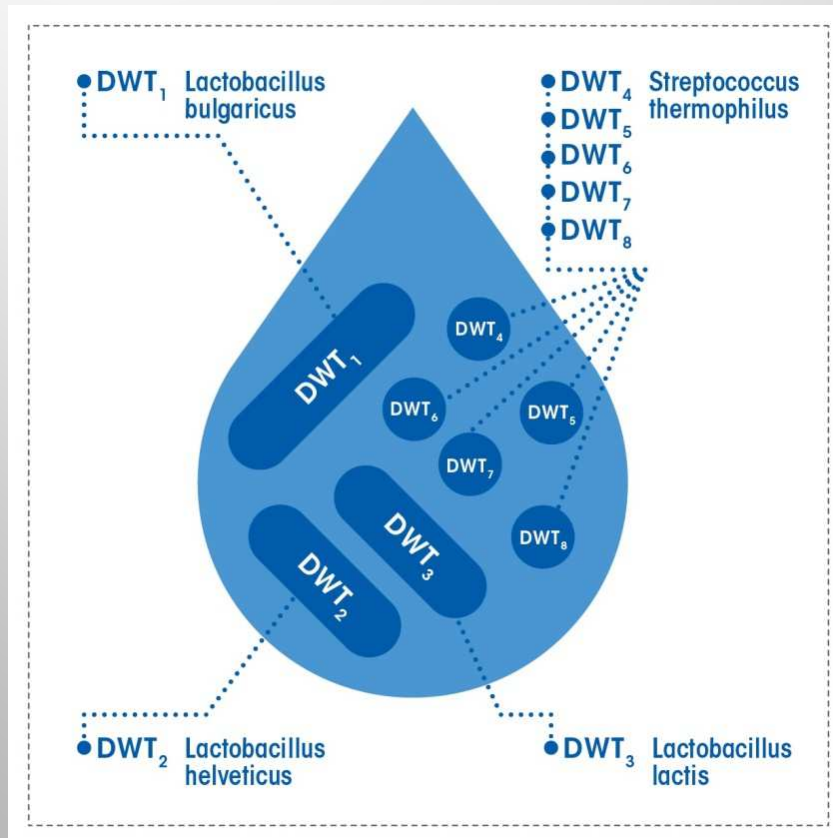
➤ Funded by Japan-Bulgarian program JICA, Hosono and Kitagawa prove antitumor effect of *Lactobacillus* in yoghurt on mice with implanted tumor cells.



# Newly found origin of *Lactobacillus Bulgaricus* on the Balkans

Until now different strains of *Lactobacillus Bulgaricus* are isolated from:

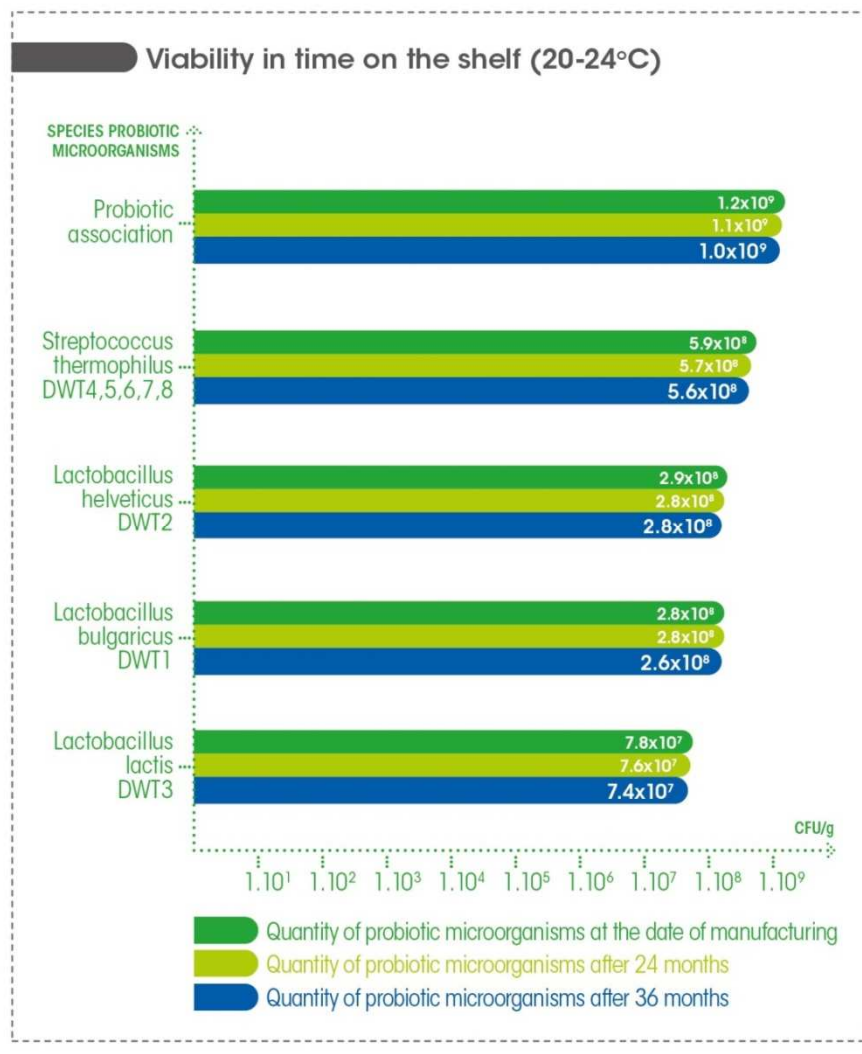
- Domestic dairy animals and their products
- Faeces of young children in Bulgaria
- Plants



For the first time in the World in 2011 N. Alexandrov and D. Petrova isolated 8 new, previously unknown, original strains of *Lactobacillus Bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus lactis* and *Streptococcus thermophilus* from spring water from the Balkan Mountains near a Thracian settlement.

# Viability of Lactobacillus Bulgaricus of water origin

The beneficial effects of probiotics depend on their viability. The studies on Lactobacillus Bulgaricus DWT1 from water origin proves its stability:



- In production process
- After rehydration when passing through the whole GIT of humans the strains of water origin survive very well under extreme experimental conditions for 7 hours in the human stomach and intestines. They colonize the colon very well.
- The strains of water origin in the probiotic formula stay alive at a room temperature (20-24°C) for more than 36 months.

# New Probiotic formula Laktera Nature®

- Using bio-coping of natural processes by modern biotechnology N. Aleksandrov and D. Petrova manage to create new probiotic formula Laktera Nature®
- Laktera Nature® is a unique combination of 8 original strains of lactic acid bacteria from water origin and natural nutrients
- Laktera Nature® has proven health claim in EU according to Regulation 432/2012 and confirmed by the Ministry of Health and Social Security in Greece. According to the claim it improves lactose digestion.



Laktera Nature® and all probiotics containing the strains form water origin are patent protected in United States of America with official Patent number US 9,131,708 B2 / Sep. 15, 2015

# Nutritional benefits of Laktera Nature®

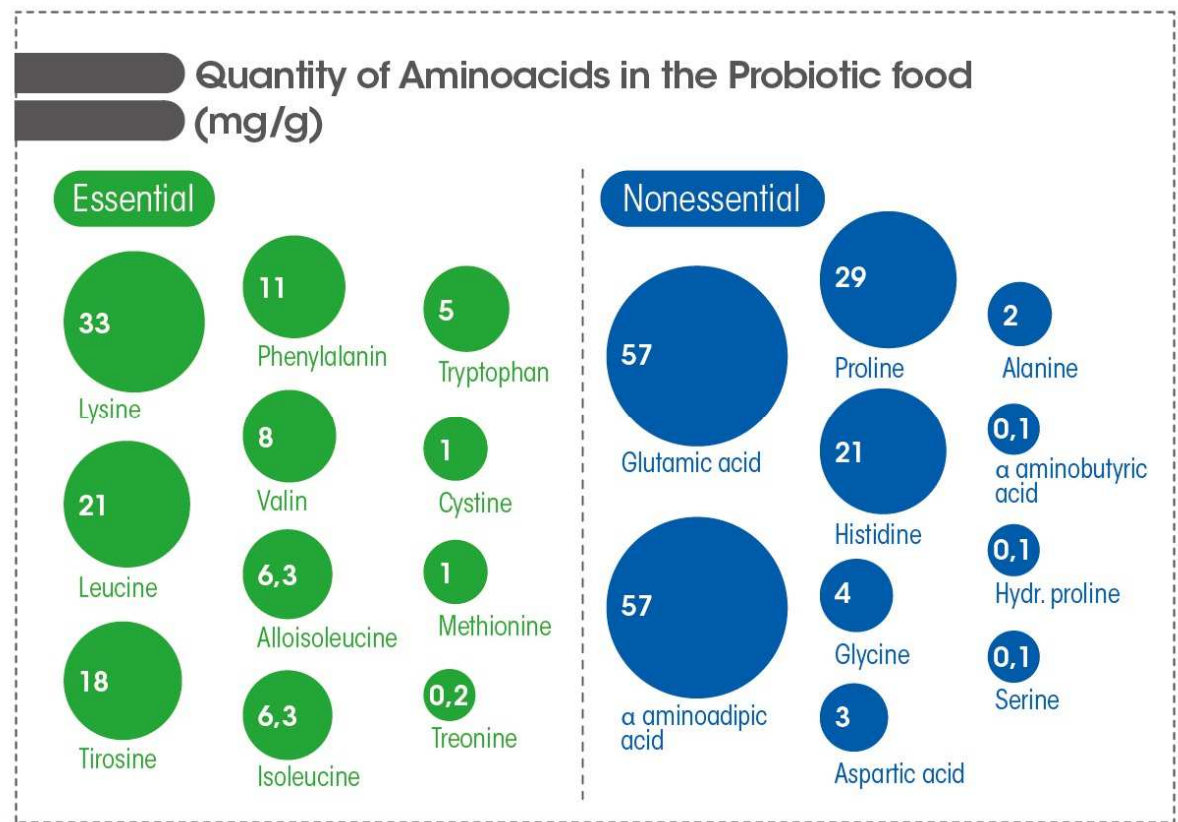
Billions CFU of *L. Bulgaricus* DWT1, *L. helveticus* DWT2, *L. lactis* DWT3 & *Streptococcus thermophilus* DWT4-8, isolated from spring water.

➤ All 21 essential and nonessential amino acids, incl. Leucine, Isoleucine and Valine called “Cocktail of life”

➤ Low molecular fatty acids – Caproic, Caprylic, Capric and Lauric and polyunsaturated fatty acids

➤ Monosaccharides – glucose, galactose; low lactose content

➤ Milk vitamins, minerals and microelements



## **Beneficial effects of Laktera Nature® – experimental and clinical researches**

✓ In 2011 N. Aleksandrov and D. Petrova prove that the probiotic formula has powerful bacteriostatic effect as they inhibit the growth of E. coli, Salmonella, Sh. flexneri, Pr. mirabilis & St. aureus and moderate bactericidal effect.

✓ In 2013 G. Aleksandrov proves that Laktera Nature® significantly improves protein absorption, overcomes hypo- and dysproteinemia and reduces complications and mortality in the early post-operative period after highly specialized abdominal surgeries.



In 2015 K. Georgiev and M. Georgieva carried out study for antiproliferative effect of Laktera Nature® against human carcinoma cell line. That study shows that in high concentration (250-1000µg/ml) Laktera Nature® inhibits the proliferation of carcinoma cell line HT-29.

# **New study of anticancer activity of Laktera Nature<sup>®</sup> on cancer cell line**

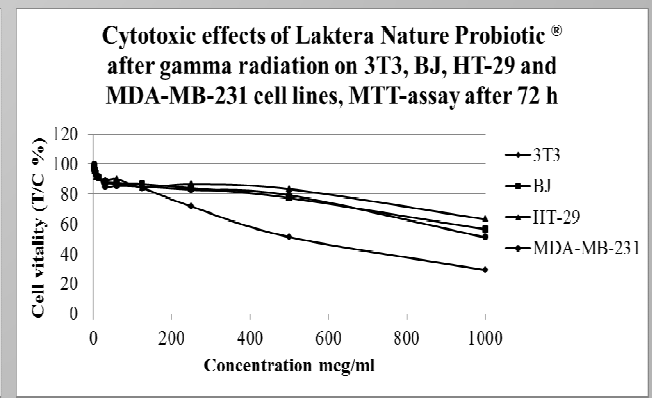
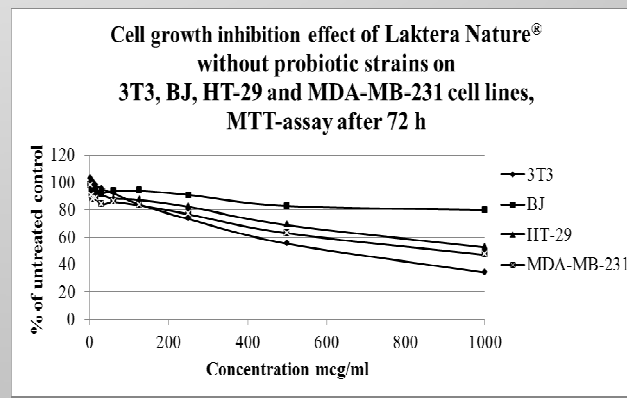
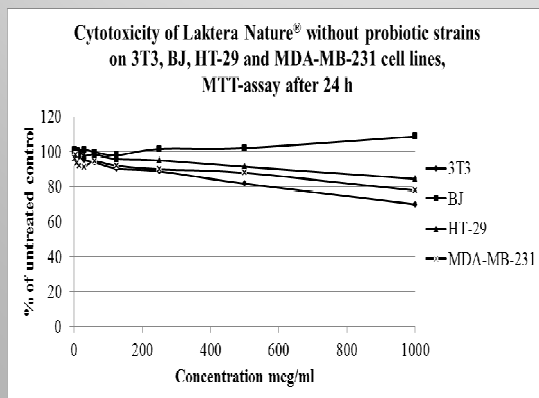
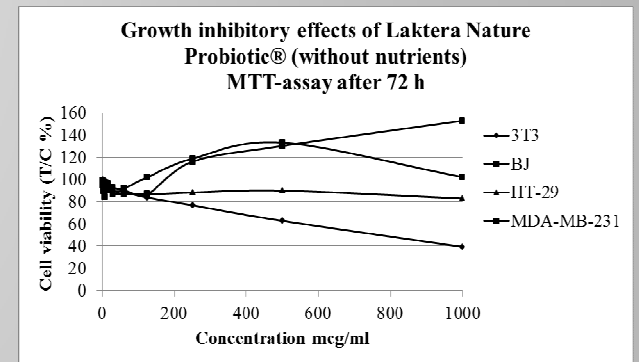
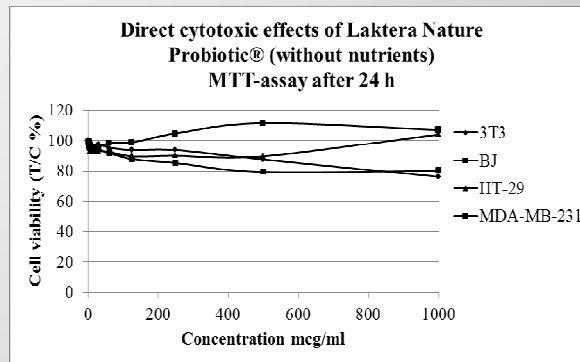
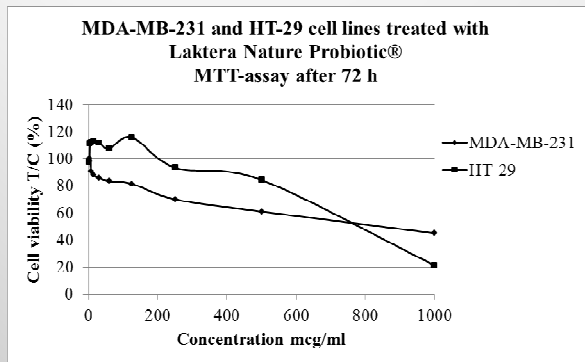
## **Materials and Methods:**

**Four types of cell lines have been used: 3T3, BJ, HT-29 и MDA-MB-231. Cytotoxicity was determined by MTT-dye reduction assay. The direct cytotoxicity of the tested cell lines, measured at the 24th hour and the antiproliferative activity, measured at the 72nd hour have been studied. Four different samples of Laktera Nature<sup>®</sup> were used:**

- sample 1 (original probiotic formula containing live cells of Lactobacillus bulgaricus DWT1 and Streptococcus thermophilus DWT4 and 21 lactic amino acids);**
- sample 2 (only the probiotic strains without the amino acids);**
- sample 3 (only the 21 lactic amino acids without the probiotic strains);**
- sample 4 (the original probiotic formula that has passed  $\gamma$ -sterilization 10 Gy, whereby the probiotic strains are killed, leaving only the cell walls)**

# New study of anticancer activity of Laktera Nature<sup>®</sup> on cancer cell line

On the figures are shown the summarized results for the 4 samples:



# New study of anticancer activity of Laktera Nature<sup>®</sup> on cancer cell line

Effect Sample	Direct cytotoxicity at the 24 <sup>th</sup> hour	Antiproliferative activity at the 72 <sup>nd</sup> hour
1	+	++
2	+	-
3	+	+
4	+	+

**Results:** the four samples show a negligible direct cytotoxicity of the tested cell lines measured at the 24th hour. Samples 3 and 4 show a slight concentration- and time-dependent cytotoxic effect.

It was interesting to observe that sample 2 (only the probiotic strains) had no the expected inhibitory effect on carcinoma growth (in that case MDA-MB-231), it even increased its growth.

Sample 1 (the original probiotic formula Laktera Nature<sup>®</sup>, containing live probiotic strains and 21 amino acids) demonstrated a significant growth inhibition of cancerous HT-29 and MDA-MB-231 cell lines with IC50 values -  $711,69 \pm 82,1$  and  $934,21 \pm 43,5$  mcg/ml respectively, measured at the 72nd hour.



# Clinical trial of effects of Laktera Nature<sup>®</sup> on patients undergone surgeries for colorectal carcinoma

➤ Clinical trial is carried out for the period 2012 – 2015 in 13 patients, aged 53 to 75 undergone surgeries for colorectal carcinoma.

➤ In all of them the histological diagnosis is: pT(1-3)N(0-1)M(0-1)V(0-1)G(1-3).

➤ All of them received 5FU + Leucovorin chemotherapy and followed by radiation therapy of total to 54 Gy.

➤ The patients are divided into 2 groups - first group (7 patients) and second group (6 patients). The patients from the first group take additionally nutritional therapy 15g/24h Laktera Nature<sup>®</sup> per os.

➤ The patients are monitored for 6 sets of indicators. The results are presented on the following table.



# Clinical trial of effects of Laktera Nature<sup>®</sup> on patients undergone surgeries for colorectal carcinoma - results

Indicators	Patients	I group (who have taken Laktera Nature <sup>®</sup> )	II group
Astheno-adynamic syndrome		29%	66%
Consumptive syndrome		14%	33%
Nausea & Vomiting		29%	83%
Frequent morbidity of viral and other infections		29%	50%
Disruption or delay in the treatment regimen due to side effects or complications		14%	50%
Mortality		14%	33%

The clinical trial shows that the nutritional therapy with Laktera Nature<sup>®</sup> significantly reduces the side effects of the chemo- and radiotherapy and in most cases allows the full courses of treatment to be carried out.

The experimental and clinical studies for the anticancer effects of Laktera Nature<sup>®</sup> continue.

# Discussion

**The original probiotic formula Laktera Nature<sup>®</sup> inhibits growth of cancerous HT-29 and MDA-MB-231 cell lines.**



**The nutritional therapy with Laktera Nature<sup>®</sup> improves quality of life and social lifestyle of the cancer patient and family.**

# Conclusion

**We suggest a new triune technological treatment regime of colorectal carcinoma:**



**Cancer surgery to remove the tumor**

**Chemo- and radiotherapy for destruction of cancer cells and decreasing the risk of recurrence and metastasis**

**Nutritional therapy with probiotic formula with Lactobacillus Bulgaricus for better health of cancer patients**

**Thanks to  
Partners Medicaire Bioscience Laboratories  
&  
MIKA Nutri-Pharma  
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**Thank you for your attention !**

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