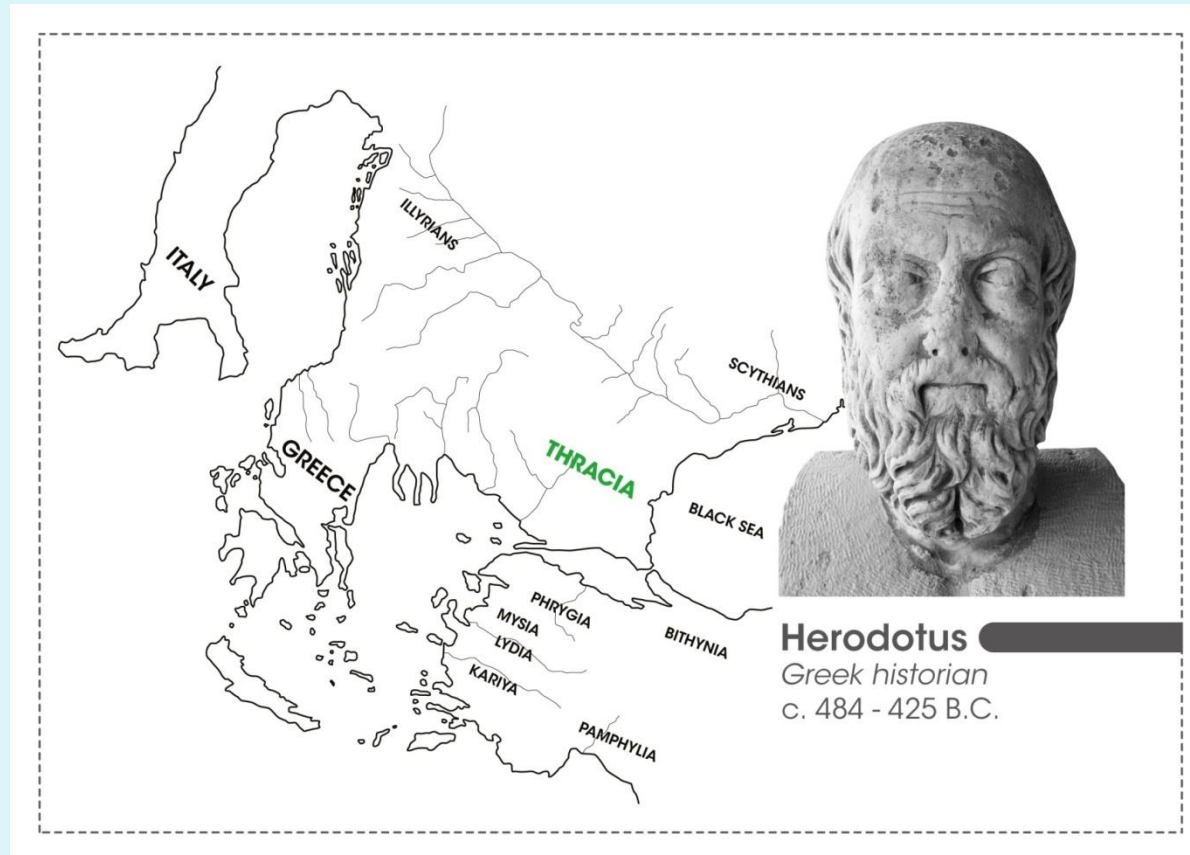
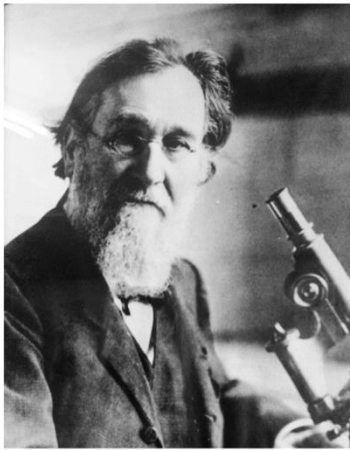


# Newly found origin of probiotics in the Balkans

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Lactic acid fermentation has been known for more than 8500 years. In 2008 around the Black Sea and southeastern Europe, prof. R. Evershed found over 2200 ceramic vessels with organic residues of lactic acid products, dated back to 6500 B.C. The first written evidence of lactic acid products were left by the Father of History, Herodotus – “...the Thracians prepared special fermented dairy foods which were a gift from their gods...”





I. Mechnikov  
1845-1916

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

## The Discovery of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*

"The longevity of the population in the Bulgarian lands is due to the daily consumption of yogurt and other products, containing lactic acid bacteria" (Mechnikov)

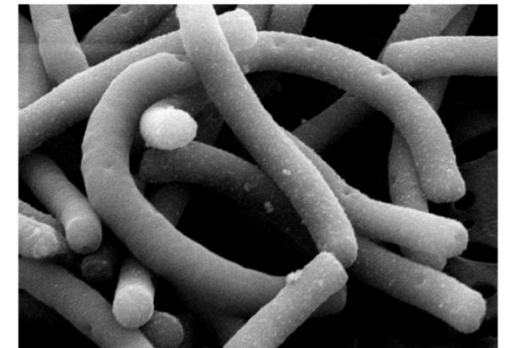
In 1905 in Geneva, the Bulgarian student St. Grigorov isolated *Lactobacillus bulgaricus* from yogurt, brought from Bulgaria.

Thus *Lactobacillus bulgaricus* became the only lactic acid microorganism bearing the name of a certain geographical territory and a certain nation.



Stamen Grigorov  
Bulgarian Biologist  
and Physician  
1878-1945

*Lactobacillus delbrueckii*  
subsp. *bulgaricus*





Typical  
Bulgarian  
Shepherd  
XIX - XX Century

## **Lactobacillus bulgaricus and Streptococcus thermophilus, origins known until now:**

- ❖ domestic dairy animals and their products
- ❖ Bulgarian infants' faeces
- ❖ plants

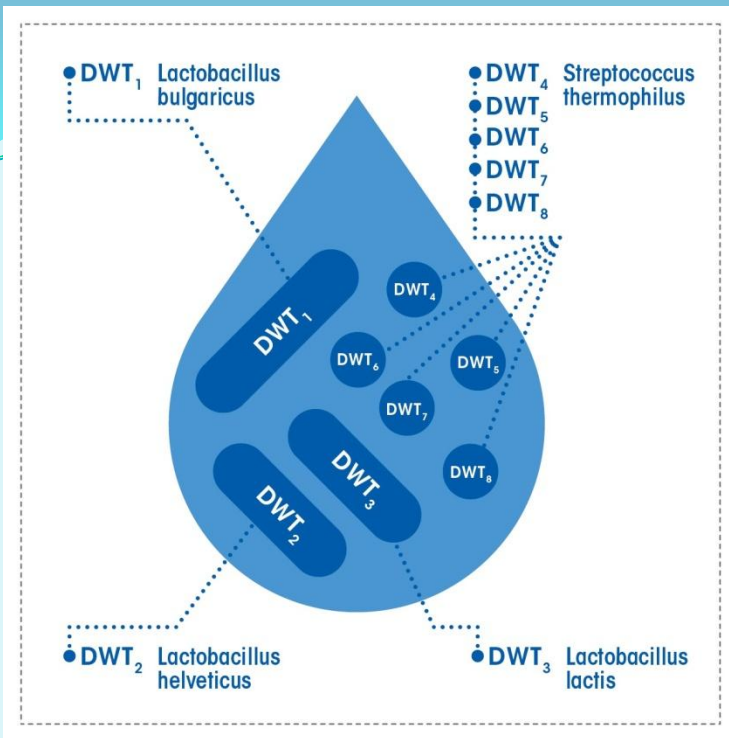
**Mihailova, Minkova, Kimura, Sasaki took 665 plant samples (priority to the plant *Cornus mas*) from 4 regions in Bulgaria and isolated 20 strains of *L. bulgaricus* & *St. thermophilus***



Lactobacillus bulgaricus isolated from plants

## NEWLY FOUND origin of *L. bulgaricus* & other lactic acid microorganisms isolated from spring water.

N. Alexandrov and D. Petrova managed to yeast ordinary pasteurized milk with spring water from the region of the Balkan, Bulgaria, near a Thracian settlement. From the obtained yoghurt they isolated 8 new unknown till now strains of *Lactobacillus Bulgaricus* and other lactic acid bacteria. The strain variety isolated from the spring water is different from the strain variety of the tree moss, soil, grass and algae.



### Composition of probiotic foods containing the lactic acid strains of water origin

- ❖ 8 strains of 4 types lactic acid microorganisms: *L. Bulgaricus*, *L. lactis*, *L. helveticus* and *Str. Thermophilus*
- ❖ 21 essential & nonessential amino acids
- ❖ low molecular fatty acids - caproic, caprylic, capric and lauric and the essential polyunsaturated fatty acids
- ❖ monosaccharides – glucose, galactose, low-lactose
- ❖ vitamins, minerals and microelements of milk

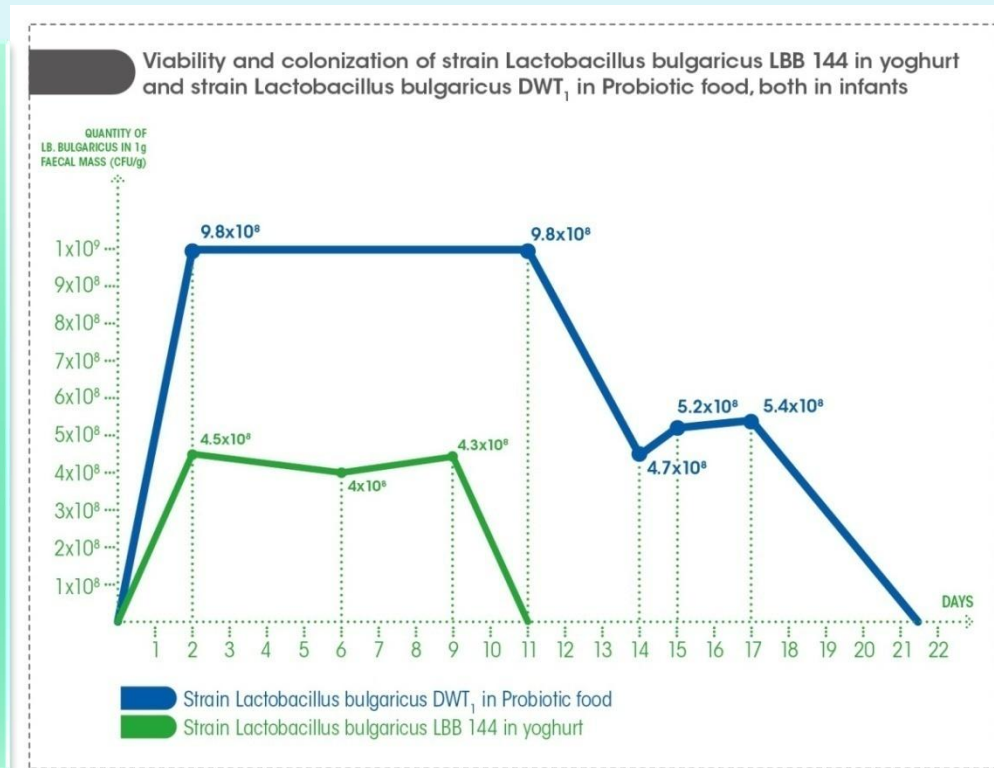


Spring in the Balkan mountain

## Viability of lactic acid strains of water origin in the probiotic food

- ❖ During LYOPHILIZATION – the strains of water origin survive freeze-drying at -45C under vacuum for 48 hours and stay alive in the obtained dry probiotic products.
- ❖ In TIME, stored at a ROOM TEMPERATURE (20 - 24C / 68 – 75F) – the strains of water origin in the probiotic food stay alive at a room temperature over 36 months.
- ❖ After REHYDRATION when passing through the WHOLE GASTROINTESTINAL TRACT – the strains have very good viability and stability after rehydration under the extreme experimental conditions for 7 hours in the human stomach and intestines.

❖ **COLONIZATION** – the study of the CFU of two strains of *L. Bulgaricus* (one of water origin and the other of yoghurt origin) in infants' feces shows that *L. Bulgaricus* DWT1 from water origin colonizes much better and has twice stronger viability in comparison with another strain of *L. Bulgaricus* in yoghurt. The number of CFU of *L. Bulgaricus* of water origin is double the number of CFU of *L. Bulgaricus* of yoghurt origin, both counted in infants' feces.

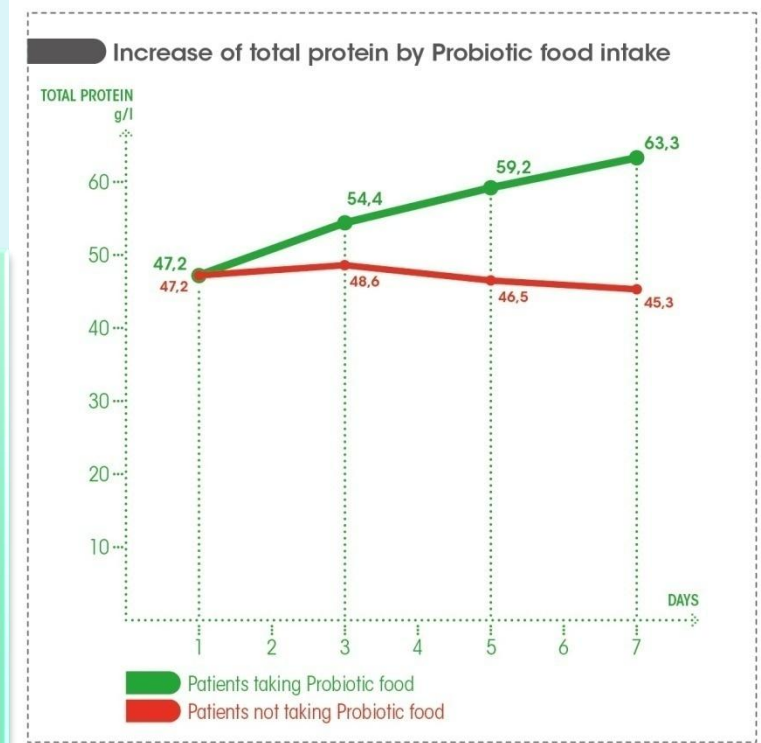


# Beneficial effects of probiotic foods containing strains of water origin

- ❖ **ANTIMICROBIAL activity** – experimental study against standardized pathogenic cultures shows that the strains have powerful bacteriostatic effect against *E. coli*, *Salmonella*, *Sh. flexneri*, *Pr. Mirabilis* and *St. aureus* and moderate bactericidal effect most against *St.aureus*.
- ❖ **On PROTEIN balance in the EARLY POST-OPERATIVE period** - double blind randomized clinical research in 75 patients selected from 1586 cases, undergone highly specialized abdominal surgeries for neoplasms

The study shows:

- ✓ Significant increase in the total protein and albumin in the group of patients administered 100g/24h rehydrated probiotic food with strains of water origin
- ✓ Amino acids in the probiotic food got hypo- and dysproteinaemias under control by the 7th day
- ✓ The significant decrease in the complications and mortality in the early post-operative period is due to the improved resorption of proteins and to the antimicrobial effect of the probiotic strains of water origin



	Number of patients	41 patients with neoplasms, who have taken probiotic food	34 patients with neoplasms, who have not taken probiotic food	1586 patients with abdominal surgery
<b>Complications</b>				
Complications		2 (4,9%)	6 (17,6%)	201 (12,7%)
Mortality		1 (2,4%)	2 (5,9%)	108 (6,8%)

## Discussion

- The newly found lactic acid strains of water origin live under changing climatic conditions and stay alive during lyophilization, in storage at 24C for 3 years. Rehydrated they survive the passage through the whole human gastrointestinal tract and colonize the colon.
- The strains of water origin have powerful bacteriostatic and moderate bactericidal effect against pathogenic microorganisms.
- Probiotic dairy food containing these strains, all natural amino acids and other nutrients significantly improves protein absorption, overcomes hypo- and dysproteinemia and reduces complications and mortality in the early post-operative period.

## Conclusion

This study undoubtedly shows the newly found water origin of *Lactobacillus bulgaricus*, *Lactobacillus hevelticus*, *Lactobacillus lactis* and *Streptococcus thermophilus*, their viability and their beneficial effect on human health.

The beneficial effects of probiotic bacteria depend on their survival in the gastrointestinal tract and their subsequent colonization in the colon, rather than their initial number in a probiotic product.

The isolation of probiotic lactic acid strains from spring water in Bulgaria brings up the question of revision of the generally accepted opinion about the origin of the lactic acid bacteria species.

**Ancient wisdom of the Thracians about the fermented lactic acid foods is transferred in the modern biotechnology by bio-coping the processes in the Nature!**



**The Phial depicts 3 whales swimming among microorganisms which swirl into the ETERNAL SPIRAL of CREATION!**

**Thracian glass phial  
"THE WATER"**  
I Century B.C.  
*Trakart Museum, Bulgaria*

