



NEW BULGARIAN UNIVERSITY

API-TEST OF LACTOBACILLI ISOLATED FROM TOP BRANDS COMMERCIAL YOGURT

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INTRODUCTION

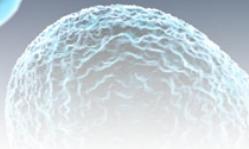
Yogurt possess numerous health beneficial effects, incl. **detoxification capability**.

Detoxification ability of yogurt is due to the lactic acid bacteria (LAB), especially on *Lactobacillus bulgaricus* which produce enzymes involved in the toxicants decomposition.

Lactic acid bacteria (LAB) are beneficial for the human health due to their **probiotic capabilities** including:

- **immune system improvement**
- **antifungal activity**
- **vitamin and enzyme production**
- as well as **bactericidal activity against pathogens**.





LACTOBACILLI DESCRIPTION (Berkeley)

- Rod shaped, long rods
- 0.5 – 1.2 /1.0-10 micrometers
- Gram positive
- Nonsporing
- Rarely motile
- Facultative anaerobes, microaerophilic
- Colonies on agar – 2-5 mm, without pigment
- Grow on rich, complex media
- Saccharolytic metabolism
- Nitrates not reduced
- Gelatine not liquified
- Catalase negative
- *cis*-Fatty acids
- Optimal growth temperature – 37-41°C



A I M

The aim of our study was to investigate the **microbial** quality of six top brand yogurt via:

- Identification of LAB isolates using **API LAB 50CHL test** (Biomerieux)
- Enumeration of viable **lactic acid bacteria count** (CFU/g yogurt)
- Lactic acid concentration in yogurt samples
- Morphological characterization of the isolates by **Scanning Electron Microscopy (SEM)**



MATERIALS AND METHODS

SAMPLES - 6 TOP YOGURT BRANDS



1. Vereya
2. Na baba
3. Elena
4. LB
5. Parshevitsa
6. Rodopeya



White color, almost no smell

MATERIALS AND METHODS

- Determination of LAB isolates - **API 50 CH test (bioMerieux, France); API software 4.0 identification**
- Count of lactic acid bacteria CFU/g yogurt – **Koch's method, MRS media**
- pH measurement
- Lactic acid concentration in yogurt samples – **1 % Phenolphthalein and 0.1 N NaOH titration/ acidic degree of Tioerner (oT)**
- Scanning Electron Microscopy of the isolates – **SEM JSM 5510 (JAPAN)**



MATERIALS AND METHODS

Yogurt content description (g per 100 g)

No	Yogurt sample	Proteins	Fat	Carbohydrates	Preservatives
1.	Vereya	3.2	3.6	4.2	no
2.	Na baba	3.2	3.6	4.1	no
3.	Elena	3.3	3.6	4.2	no
4.	LB	3.2	3.6	4.2	no
5.	Parshevitza	3.2	3.6	4.2	no
6.	Rodopeia	3.2	3.6	4.2	no



*All manufacturers declared yogurt do not contain any milk powder



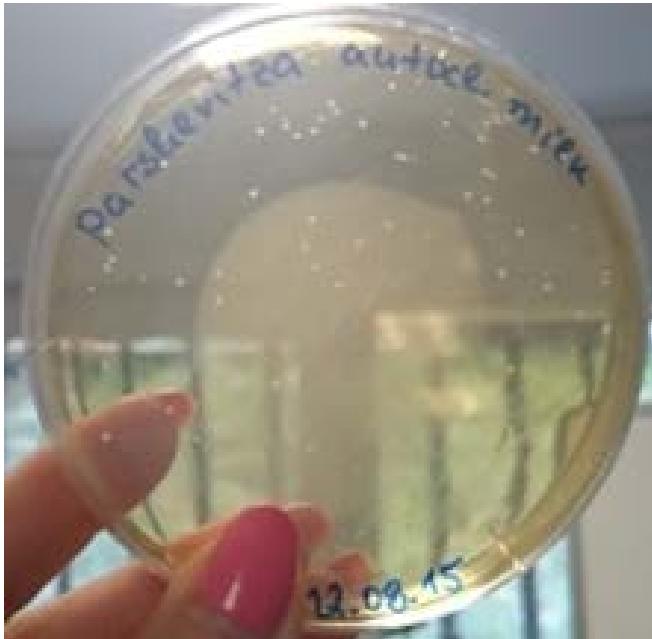
R E S U L T S



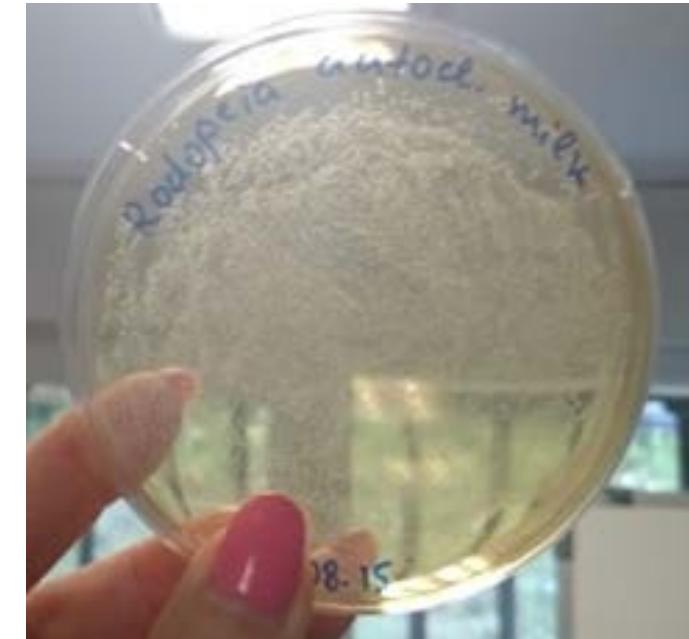
LACTIC ACID BACTERIA ISOLATES



Control



Parshevitsa isolates



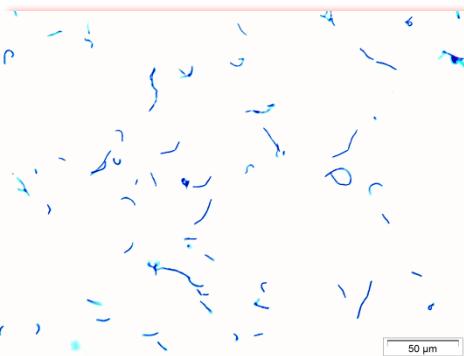
Rodopeya isolates



LACTIC ACID BACTERIA ISOLATES



LIGHT MICROSCOPY



“Na baba 2” isolate



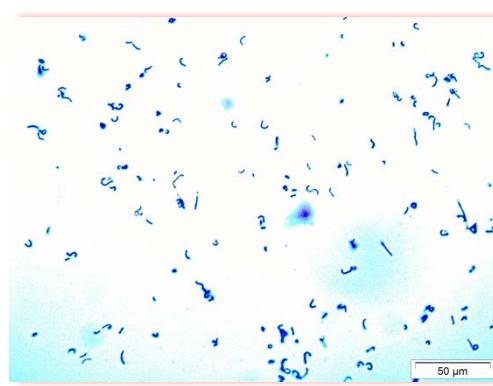
“Elena” isolate



“Parshevitsa” isolate



“LB” isolate



“Na baba 1” isolate



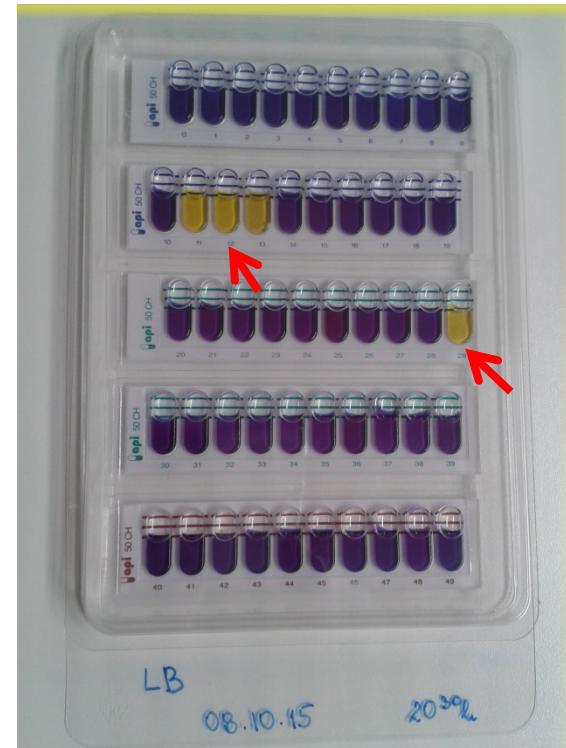
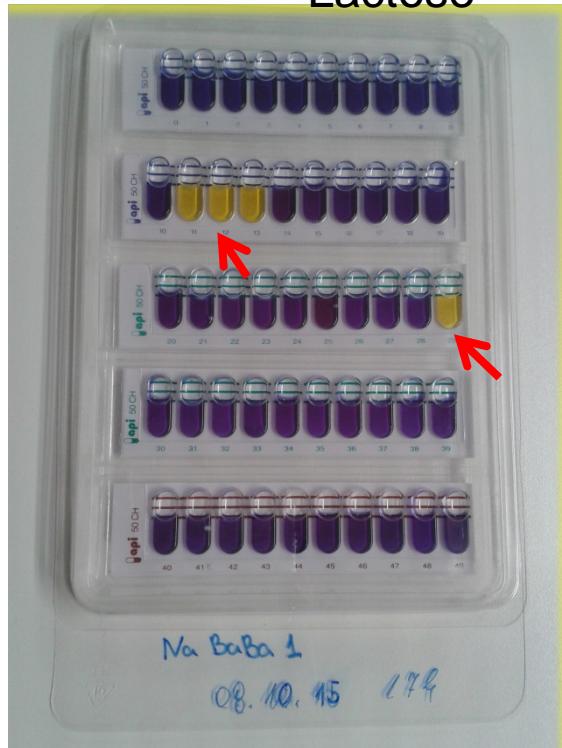
“Vereia” isolate

DEGRADATION OF 50 METABOLITES WAS ANALYZED

glycerol, erythrol, D-and L-arabinose, D-ribose, D- and L-xylose, D-adonitol, methyl-beta-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, D-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-alpha D-mannopyranoside, methyl-alpha D-glucopyranoside, N-acetylglucosamin, amygdalin, arbutin, esculin/ferric citrate, salicin, D-celllobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, inulin, D-melizitose, D-rafinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, potassium guconate, potassium 2-ketoguconate, potassium 5-ketoguconate ribose

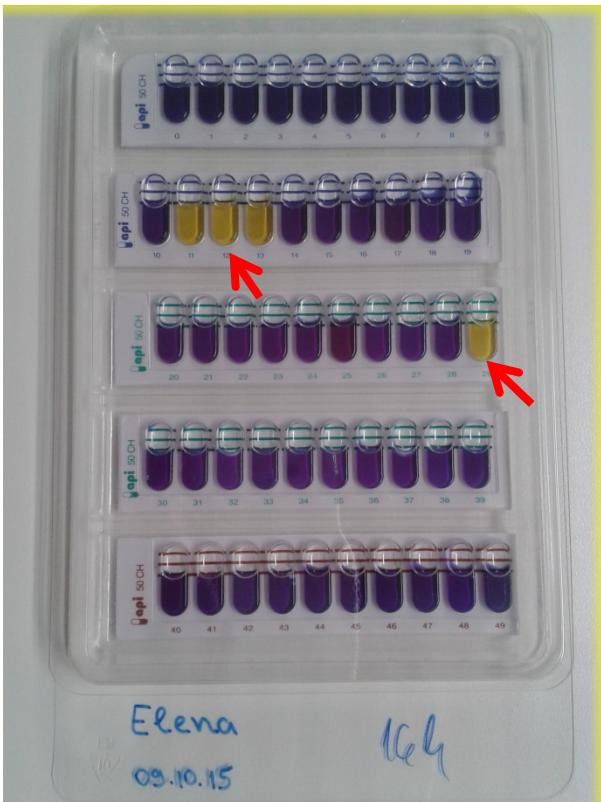


Glucose
Fructose
Mannose
Lactose



Glucose
Fructose
Mannose
Lactose

API CH 50 TEST



API CH 50 TEST

BIOCHEMISTRY



API LAB SOFTWARE 4.0 IDENTIFICATION

Na baba 1 isolate: *Lactobacillus delbrueckii* subsp. *bulgaricus*, Affiliation - 98.8%

Na baba 2 isolate: *Lactobacillus delbrueckii* subsp. *bulgaricus*, Affiliation - 98.8%

LB isolate- *Lactobacillus delbrueckii* subsp. *Bulgaricus*, Affiliation - 98.8%

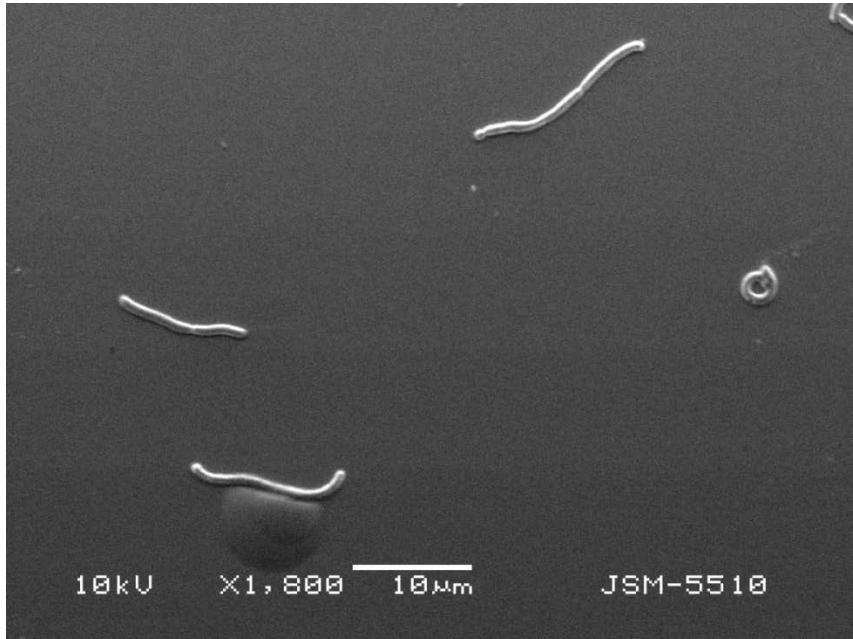
Elena isolate - *Lactobacillus delbrueckii* subsp. *bulgaricus* Affiliation - 98.8%

Purchevitca izolate: Doubtfull profile - *Lactococcus lactis* subsp. *cremoris* (0.8%) or *Aerococcus viridans* (99.2% identity)Additional tests needed - Genciobiose- 99%(+); Saccharose (16)%

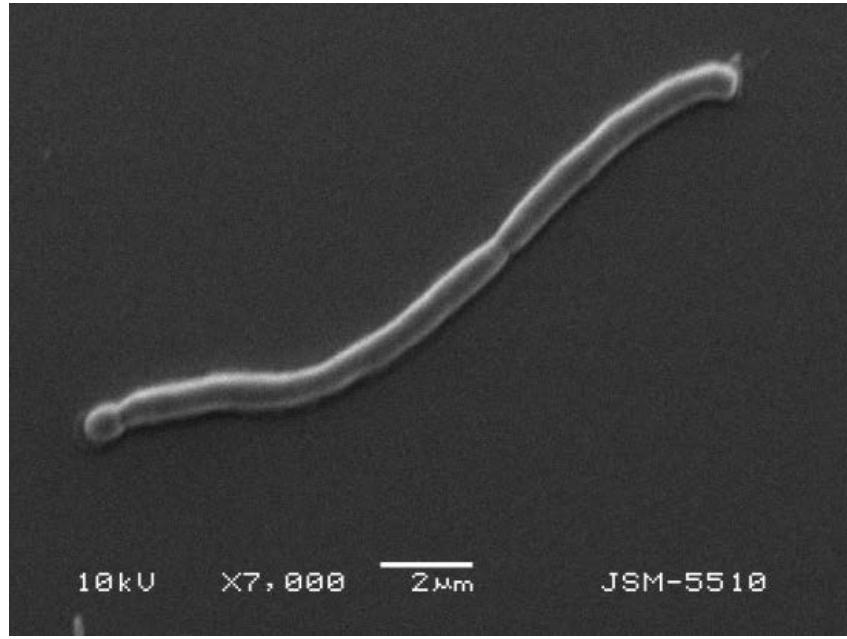
Vereya isolate: *Lactobacillus paracasei* *paracasei* 1: Doubtfull profile (98.5% and low T-0,48)



SCANNING ELECTRON MICROSCOPY OF LAB ISOLATES



x 1 800

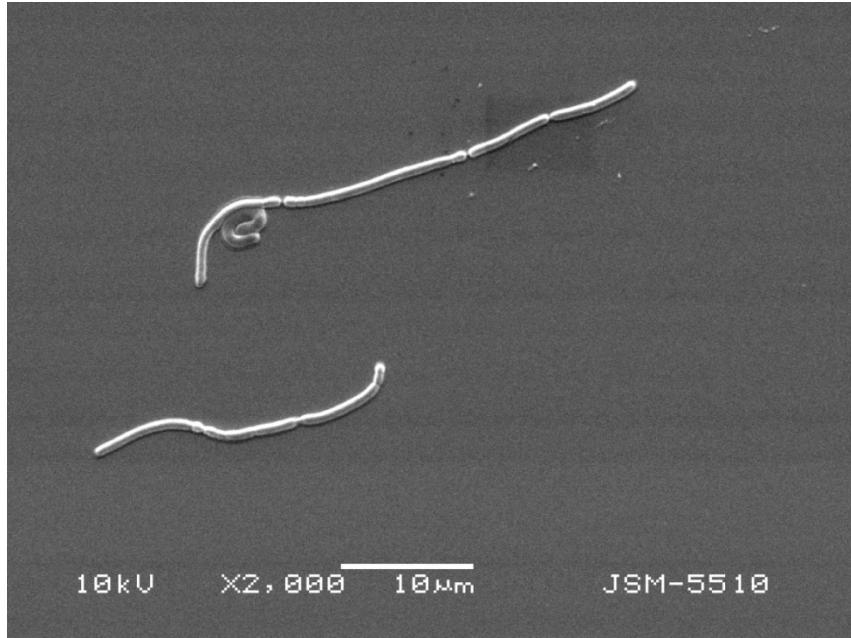


x 7 000

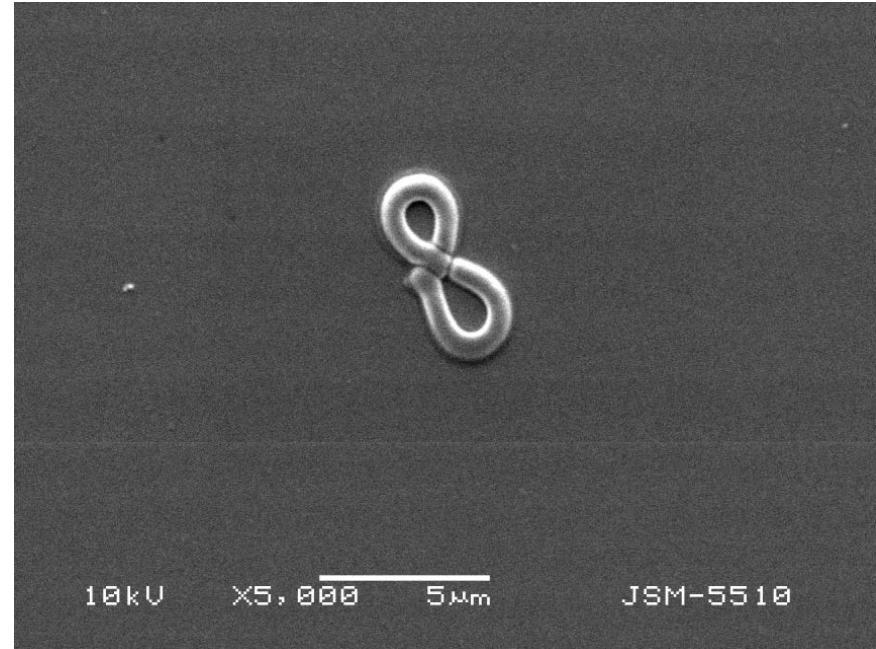
“Na baba 1” isolate



SCANNING ELECTRON MICROSCOPY OF LAB ISOLATES



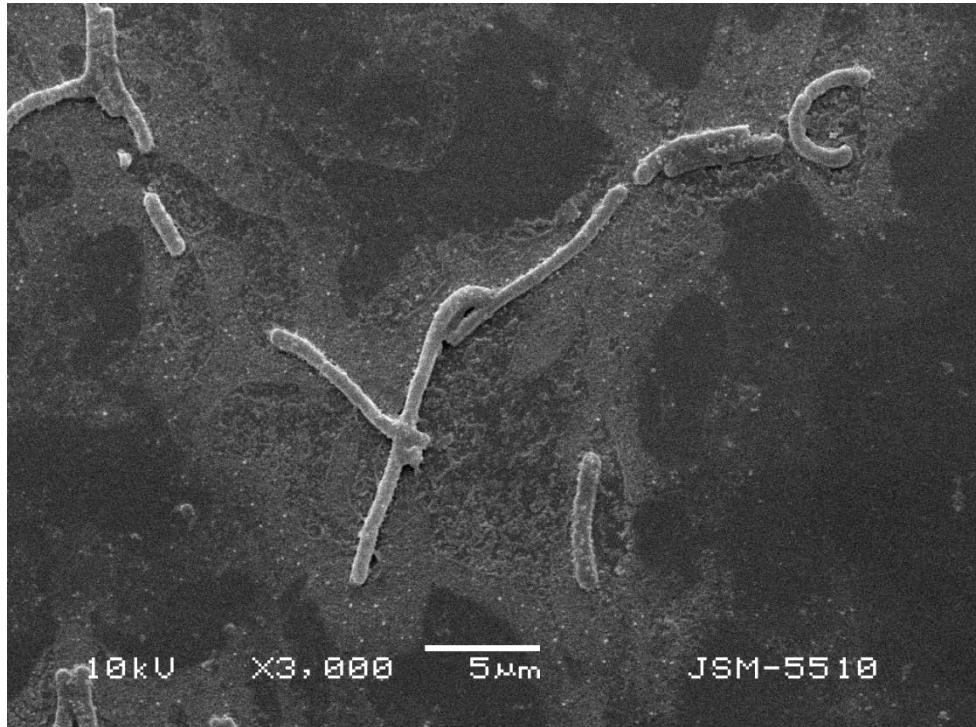
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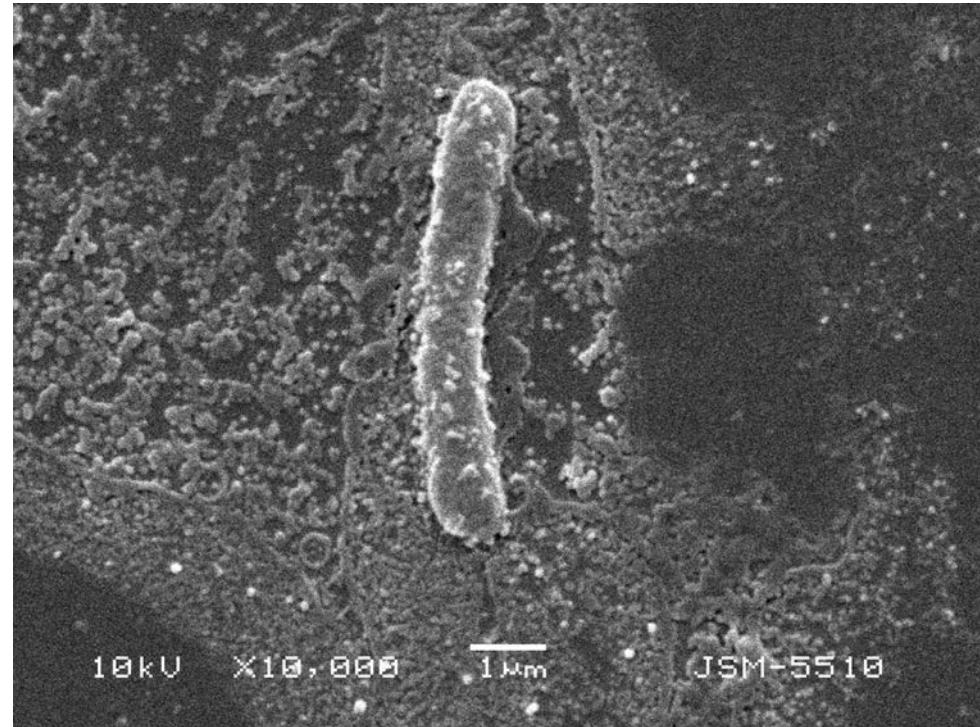
x 5 000



SCANNING ELECTRON MICROSCOPY OF LAB ISOLATES



x 3 000

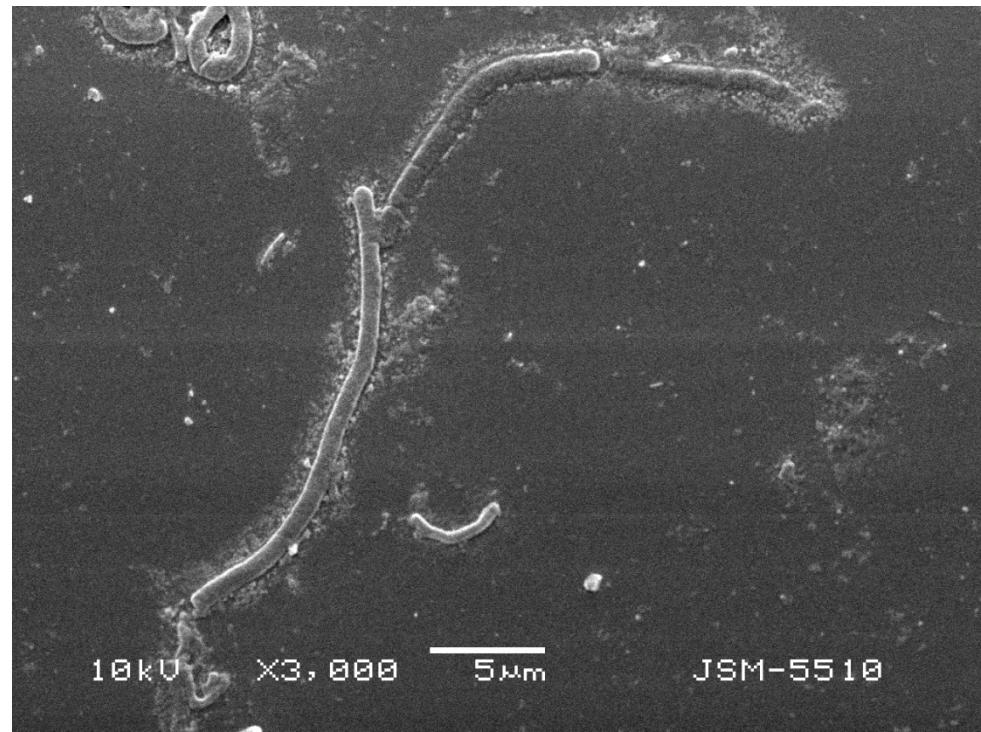
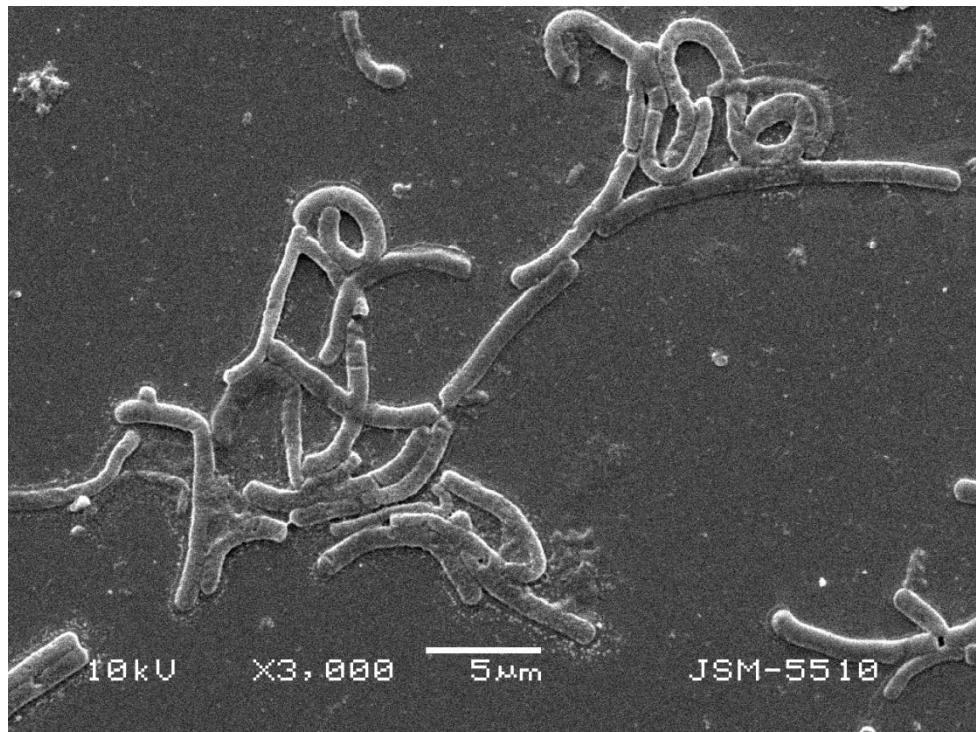


x 10 000

“Na baba 2” isolate



SCANNING ELECTRON MICROSCOPY OF LAB ISOLATES



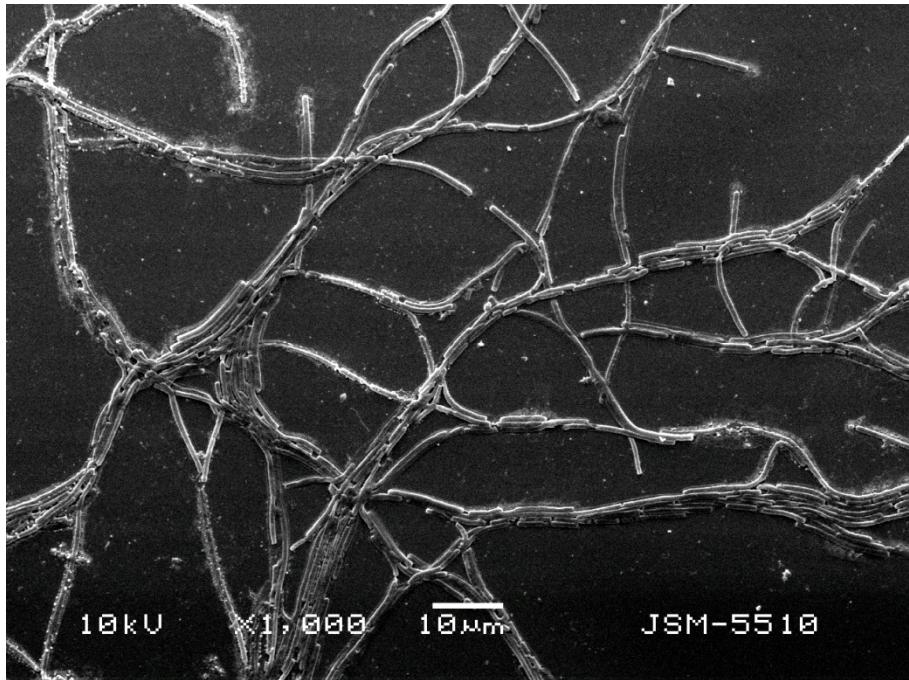
x 3 000

x 3 000

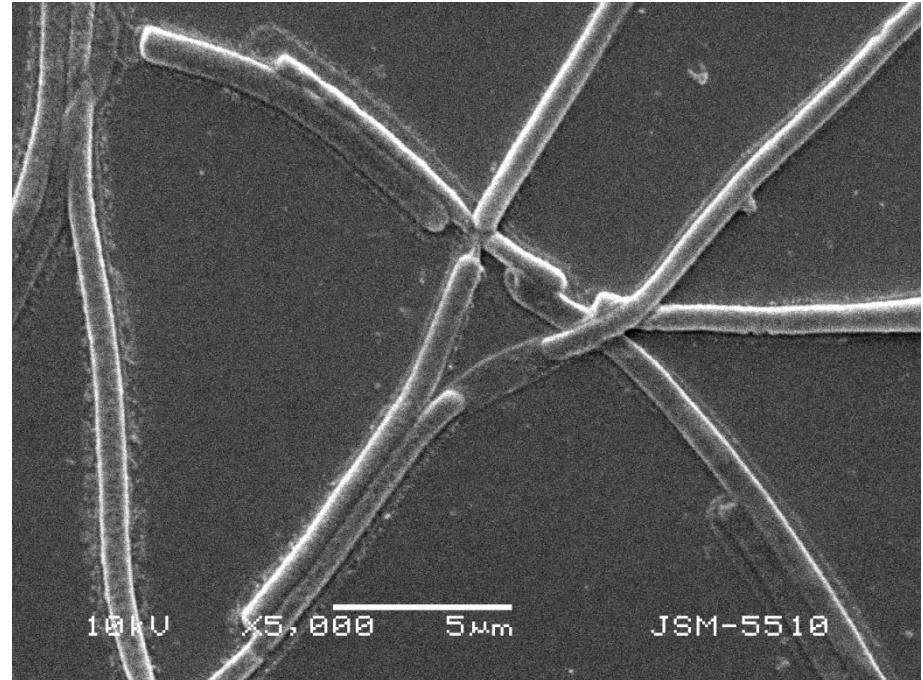
“LB” isolate



SCANNING ELECTRON MICROSCOPY OF LAB ISOLATES



x 1 000



x 5 000

“ELENA” isolate



LACTIC ACID BACTERIA (LAB) COUNT (CFU/g)

No	Yogurt sample	Lactobacilli CFU/g yogurt
1.	Vereya	2.6×10^6
2.	Na baba	3.4×10^6
3.	Elena	0.7×10^6
4.	LB	7.1×10^7
5.	Parshevitza	2.8×10^7
6.	Rodopeia	4.0×10^6

According the Bulgarian State Standard (BDS) the count of *L. bulgaricus* per gram yogurt should be **1×10^6 to 1×10^7 CFU/g.**



pH MEASUREMENT

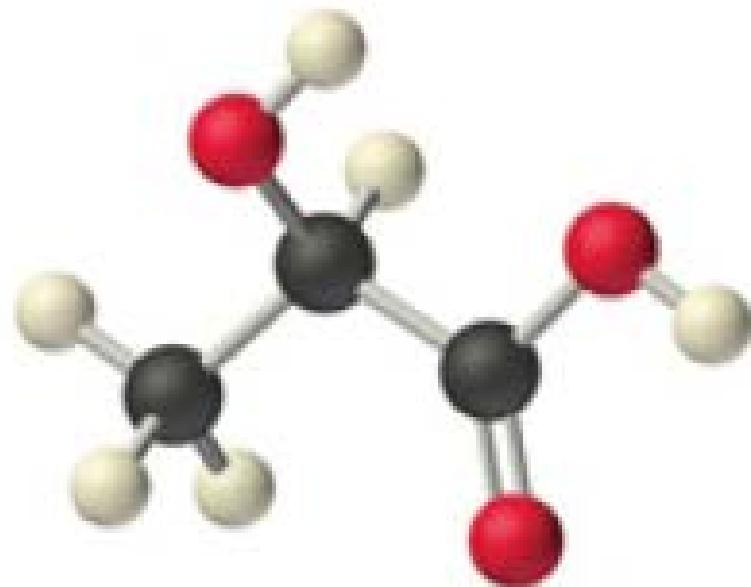
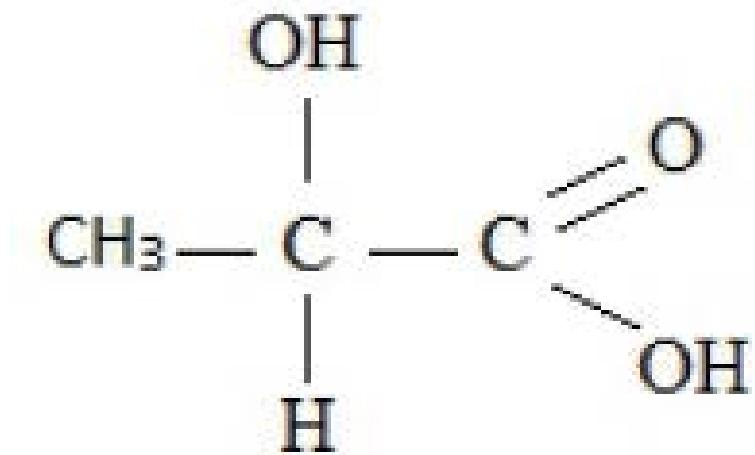
No	Yogurt sample	pH value
1.	Vereya	4.4
2.	Na baba	4.4
3.	Elena	4.5
4.	LB	4.4
5.	Parshevitza	4.5
6.	Rodopeia	4.5

Optimal pH for the members of genus Lactobacillus is 4.4- 4.6.



pH 4.4 - 4.5

LACTIC ACID



**IXth National Scientific Conference of EFSA Focal centre
in Bulgaria, Hissar, 24-25 October, 2016**

G. Satchanska

LACTIC ACID CONCENTRATION IN YOGURT

Sample	Brand	oT	%lactic acid
S1	Vereja	142	12,78
S2	Na Baba	137	12,33
S3	Elena	142	12,78

S10	Elbi	183	16,47
S11	Purshevica	117	10,53
S12	Rodopeia	127	11,43



CONCLUSIONS

1. API CH 50 test showed LAB isolates of Na baba, LB and Elena yogurt belong to *Lactobacillus delbrueckii subsp. bulgaricus*, degrading 4 carbohydrates. Parshevitsa and Vereia yogurt showed doubtful profile.
2. Six investigated yogurt samples cover the Bulgarian state standard: lactobacteria exceed 1×10^6 CFU/g except Elena yogurt (0.7×10^6). The highest number of lactobacilli was enumerated in LB yogurt
3. Highest lactic acid concentration was demonstrated in LB yogurt
4. SEM showed typical for *L. bulgaricus* morphology





European Food Safety Authority