BaSeFood
(G.A. n° 227118)

Sustainable exploitation of bioactive components from the Black Sea Area traditional foods

D 2.5a:

FINAL REPORT OF THE MICROBIOLOGICAL CHARACTERISATION OF TYPICAL BSAC FOODS

Olga Levchuk, Mariia Mudryk, Viktor Petrov, Anzhela Dolgikh, Ivan Kutchak, Andriy Pauk, *and* Nadiya Boyko (UzhNU)

Contributors of food samples: Iordanka Alexieva and TanaSapundzhieva (UFT),
Dmitry Karpenko (MSUFP), Osman Hayran and Bike Kocaoglu (YEDITEPE)

WP Leaders: Helena Costa (INSA) and Paul Finglas (IFR)

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CONTENT

INTRODUCTION

The background to this task lies in *the substantial lack of information on microbiological food safety of some traditional foods from Black Sea area countries (BSAC) and consequently the need to study this area to assure the overall safety of these foods* (see**D2.2**).

In this report, the results of microbial estimation of selected foods from countries of Black Sea Region within BaSeFood project are presented. The major plant components used asfoods ingredients have been tested microbiologically in order to determinetheir contamination (association) with three different groups of bacteria: **A** – beneficial, **B1** – detrimental, but mainly of environment origin and very often belonged to potentially pathogenic bacteria,and **B2**– themost dangerous group, so-called food-borne (strong) pathogens,which are usually of human origin.

There are two key tasks included in the project. The first task was*to use rather "routine"detection methods for key-microorganisms which are generally accepted (defined) as indicators (determinants) ofthe safetyof the selected foods*. The second was *to identifythe keymicroorganisms in traditional foods and in raw (plant) material which can affect (either positively or negatively)the quality of the selected traditional foods*.Correspondingly,in this task each partner from BSAC provided the selected food samples/raw materials specifiedin **WP1** and **WP4**which were tested microbiologically in order to identifythe "food safety" or"food/quality" issues.

The possiblerole of the majorisolates in modifying the nature and/or availability of biological-active compounds in traditional foods is reported along with theanalytical data (see **D2.3**); however, these results will be interpreted here as will the detrimental influence of BAS in plants having specific bacterial persistence.

The traditionalfoods and plant samples wereinvestigated according to the DoWusingwell-known bacteriologicalassays and International and National Standards for themicrobial analysis of foods[1 – 23].Some of these methods (for example,Hazard Analysis Critical Control Point, HACCP) are used mainly by food-related industries as a meansto identify critical control points where lack of compliance or failure to meet standards can introduce food-borne illness or product contamination. Such methods area qualitative, rather than a quantitative, tool.

In orderto standardise the types of assay required to fulfill this task,the different methods have been adapted and harmonized, and specific methodologiesdeveloped to analyse the samples. These modifiedmethodologieswere initiallyverified and validatedintriplicateafter which the**unified methodological manual**was proposed. According to this document all the samples of raw materials(plants) and selected traditional foods (listed in **Tables 1 and 2**) werehomogenised, weighed and titrated in PBS for the quantitative assay, and then platedin 10 μl samples at dilutions of 10, 10^3, 10^5, 10^7, 10^9on selective and chromogenic media (**Figs. 1, 2, pp. 6, 9-11**).

Identification of the isolated targeted microorganism species (**Table 3**) was conductedusing URI select tests, OXI-tests, API test systems for rapid biochemical identification – API 32E, API NH, API 20 C AUX, API STREP, API STAPH, API 20 NE, API 50 CH, API 50 CHB, API CANDIDA, API CORYNE, (BioMérieux, France), and ANAERO test 23 and ENTERO-test 24 PLIVA (Lachema Diagnostika s.r.o, Czech Republic); serological identifications were performed additionally usingPAST Staphy- and Strep- Latex-tests (Bio-Rad, USA).

To determine the targeted microorganisms, complex chromogenic media (bioMérieux, France) wasused: Ottaviani Agosti Agar (for detection and enumeration of *Listeria spp.* and *L. monocytogenes*), SM ID 2 (for Salmonella detection), COLI ID (for detection of *E. coli* and total coliforms), O157:H7 ID (for detection of *E. coli* O157:H7), CampyFood ID (for *Campylobacter spp.* detection). Raw materials wereadditionally tested on the persistence of yeasts and molds (Oxoid, Remel RapID™ Yeast Plus identification panel).

Table 1. Prioritised traditional foods from BSAC, divided into food groups.

Food Groups	Traditional foods		Countries
	Food name (English)	Food name (national language)	
1. Cereals and cereal-based foods	Sour rye bread	Хліб житній	Ukraine
	Buckwheat porridge crumbly	Каша гречневая рассыпчатая	Russian Federation
	Cornmeal mush	Mămăligă	Romania
	Tsiteli Doli Bread	Makhobeliani dolis puri	Georgia
	Baked layers of pastry stuffed with pumpkin	Tikvenik	Bulgaria
	Bulgur pilaf	Bulgur pilavı	Turkey
2. Vegetables and vegetable-based foods	Ukrainian borscht	Борщ український пісний	Ukraine
	Transcarpathian green borscht	Zelenyj borshch Zakarpats'kyj	Ukraine
	Vegetable *okroshka*	Овощная окрошка	Russian Federation
	Nettle sour soup	Ciorbă de urzici	Romania
	Nettles with walnut sauce	Chinchris mkhali nigvzit	Georgia
	Rhodopian dried beans	Rodopski fasul	Bulgaria
	Kale soup	Kara lahana corbasi	Turkey
3. Fruit or fruit-based foods	*Uzvar*	Узвар	Ukraine
	Watermelon juice	Арбузный сок	Russian Federation
	Plum jam	Magiun de prune	Romania
	Churchkhela	Churchkhela	Georgia
	Rose jam	Dko ot rozi	Bulgaria
	Fruit of the evergreen cherry laurel	Karayemiş	Turkey
4. Oilseeds or oilseed products	Roasted sunflower seeds	Smazhene nasinnya	Ukraine
	Mustard oil	Горчичное масло	Russian Federation
	Flax oil	Selis zeti	Georgia
	Tahini Halva	Tahan Halva	Bulgaria
5. Herbs, spices and aromatic plants	*Pomazanka*	Pomazanka	Ukraine
	Herbal dish	Mâncărică de verdeaţă	Romania
	Wild plum sauce	Tkhemlis satsebeli	Georgia
	Mursal tea	Mursalski chai	Bulgaria
	Black tea	Çay	Turkey
6. Low or non-alcoholic fermented foods and beverages of plant origin	Sauerkraut	Капуста білокачанна квашена	Ukraine
	Kvass southern	Квас южный	Russian Federation
	Elderberry soft drink	Socata	Romania
	Millet ale	Boza	Bulgaria
	Sautéed pickled green beans	Fasulye turşusu kavurması	Turkey

Table 2. Major plant ingredients of traditional foods from BSAC[1)]

Food Groups	Samples		Countries
	Food name (English)	Plants	
1. Cereals and cereal-based foods	Sour rye bread	ryeflour	Ukraine
	Buckwheat porridge crumbly	buckwheat	Russian Federation
	Cornmeal mush	corngrits	Romania
	Tsiteli Doli Bread	wheat flour (Tsiteli Doli variety), Makhobeli seeds	Georgia
	Baked layers of pastry stuffed with pumpkin	pumpkin, cinnamon, wheat flour, walnuts	Bulgaria
	Bulgur pilaf	siyez bulgur, tomato, green and red pepper, onion, parsley	Turkey
2. Vegetables and vegetable-based foods	Ukrainian borsch	beet, tomato, potato, dill, parsley green, parsley root, beans, cabbage	Ukraine
	Transcarpathian green borsch	sorrel, potato, carrot, onion, parsley, garlic, dill	Ukraine
	Vegetable *okroshka*	cucumbers, green onion, potato, carrot, turnip, radish, dill	Russian Federation
	Nettle sour soup	nettle, green onions, carrot, celery roots, rice	Romania
	Nettles with walnut sauce	nettle, walnut	Georgia
	Rhodopian dried beans	rhodopean/smilyan beans, dried, savoury, carrot, onion, tomatoes, flour, hot pepper	Bulgaria
	Kale soup	kales, onion, black pepper, tomatoes, corn grits	Turkey
3. Fruit or fruit-based foods	*Uzvar*	apples, pears, plums – fresh and dried	Ukraine
	Watermelon juice	water melon	Russian Federation
	Plum jam	plums	Romania
	Churchkhela	grape juice, fresh fruits, walnut	Georgia
	Rose jam	rose petals, dried and fresh	Bulgaria
	Fruit of the evergreen cherry laurel	cherry laurel fruits	Turkey
4. Oilseeds or oilseed products	Roasted sunflower seeds	sunflower seeds	Ukraine
	Mustard oil	mustard seeds	Russian Federation
	Flax oil	flax seeds	Georgia
	TahiniHalva (*Tahan* halva)	sunflower seeds, Good King Henry roots	Bulgaria
5. Herbs, spices and aromatic plants	*Pomazanka*	dill, garlic	Ukraine
	Herbal dish	basil, dill, parsley, mint, sage leaves, onion, tomato, black and red pepper, wheat, corn flour, cherry leaves	Romania
	Wild plum sauce	wild plums red and green, garlic, red pepper, coriander, fennel, pennyroyal	Georgia
	Mursal tea	*Sideritis scardica*	Bulgaria
	Black tea	black tea, green tea	Turkey
6. Low or nonalcoholic fermented foods and beverages of plant origin	Sauerkraut	cabbage, carrot	Ukraine
	Kvass southern	rye bread, juniper berries, raisins	Russian Federation
	Elderberry soft drink	lemon, elderberry flowers*	Romania
	Millet ale	wheat, barley, grape juice, honey, sugar	Bulgaria
	Sautéed pickled green beans	green beans , onion, garlic, tomatoes, rice, black pepper	Turkey

[1)] Latin scientific (systematical) names of plants are provided in D1.3.

Table 3. Targeted groups[2)] of microorganisms in sub-task 2.4.1.

Beneficial, (A)	**Saprophytic/epiphytic, (B1, a) Environmental origin**	**Potentially pathogenic, (B1, b) Human origin**	**Food-borne pathogens, (B2)**
Enterobacteriaceae: *E. coli* (lactose +)	Enterobacteriaceae: *Erwinia spp.* Others	Enterobacteriaceae: *E. coli* (lactose -) *Citrobacter spp.* *Klebsiella spp.* *Enterobacter spp.* *Enterococcus spp.* *Serratia spp.* *Proteus spp.* Others	Enterobacteriaceae: *E. coli (EPEC)* *Salmonella enterica* *Shigella dysenteriae* *S. flexneri* *E. coliO157:H7*
LAB: Lactobacillus *L. acidophilus* *L. salivarius* *L. fermentum* Others	*Pseudomonas fluorescence* *Xanthomonas campestris* Other pigmented microorganisms	*Pseudomonas aeruginosa*	
Bifidobacteria: *Bifidobacterium bifidum*			*Listeria monocytogenes*
Bacillus: *B. subtilis* *B. licheniformis*	Bacillus: *Bacillus licheniformis*	Bacillus: Others	
Enterococcus: *Enterococcus faecalis* *E. faecium*	*Non-pathogenic species of Staphylococcus – coagulase negative staphylococci (CNS)* *Streptococcus spp.* *Micrococcus spp.*	*Staphylococcus aureus,* *Streptococcus pyogenes*	
Saccharomyces: *Saccharomyces boulardii* *S. cerevisiae*	*Aspergillus spp.* *Penicillium spp.* *Fusarium spp.* Others (all of cultivated)	*Candida spp.*	
Bacteroides distasonis			*Campylobacter jejuni*
Clostridium: *Clostridium buturicum*	Clostridium: *Clostridium perfringens*		Clostridium: *Clostridium botulinum*

[2)] *- Explanation of screening of the targeted microbial key-species within following systematic groups:*

1) *Staphylococcus and Streptococcus* as potentially dangerous human pathogens;
2) *Enterobacteriaceae* – all representatives; including commensal (normal, nonpathogenic species), potentially pathogenic species and strong pathogens)*;*
3) *Pseudomonadaceae* – two groups only – plants-associated epiphytic bacteria and human-associated potentially pathogenic bacteria;
4) *Lactic acid bacteria (LAB) and Bifidobacteria* as presumably (according to definition) beneficial microorganisms;
5) Bacillus spp. as widely presented contaminant microorganism including probiotic, and potentially pathogenic species;
6) *Clostridium group* – as widely distributed anaerobes with specific attention to toxin producing species *(Clostridium botulinum)* and sanitary indicative microorganism *Clostridium perfringens* human pathogen *Clostridium perfringens;*
7) *Bacteroides* as commensal bacteria with not well defined roles in some human diseases*;*
8) Microscopic fungi with particular attention to*: 1) Saccharomyces* as beneficial species and others*: 2)* of environment *Aspergillus spp. Penicillium spp. Fusarium or 3)* human origin – *Candida;*
9) *Salmonella, Shigella, Listeria and Campylobacter* – as "typical" food-borne pathogens*;*
10) Group of pigmented bacteria – presented mainly by epiphytic microorganisms.

MATERIALS AND METHODS

The typical protocol of the microbial investigation of food products (all the BSAC samples) were then usedin task 2.4.2.

All the samples were randomly selected from different sources (from city and street markets, yards – plants;families – home-made foods and industrial processed products – purchased from restaurants). One sample was collected in triplicate and then estimated microbiologically. Each sample was initially divided intothree parts – for one qualitative and two quantitative tests.

Qualitative assay

A fingerprint method was used to isolate the targeted groups of microorganisms by direct plating of all the plant samples on media containing different nutrients: Nutrient Agar and Broth, Chapek-Dox Agar (Broth), Schaedler Agar (Broth), Blaurock medium, Wilkins-Chalgren Agar (Broth), Endo (MacConkey), Lactobacilli MRS Agar (Broth), Salmonella/Shigella Agar (Broth), Bismuth-Sulfite Agar, Yolk-salt Agar, Sabouraud Agar, Enterococcus Agar, Potato Agar, URI-select medium, Clostridium Agar (Broth), Starch Agar, Rice Agar, Blood Agar.

Quantitative analysis

One gram of theplant ingredient or ready-to-eat meal was dissolvedin 10 ml sterile PBS (10^{-1}) and the appropriateserial dilutions were then plated in an amount of 10 µl and colony-forming units per ml, CFU/ml (N) wascalculated by the formula:

N = A · B · C, where:**N** – number of microorganisms in 1 ml of samples, CFU/ml;**A** – number of colonies on the media;**B** – corresponding dilution coefficient, and **C** – inoculating coefficient (for 100 µl – 10; for 10 µl - 100):

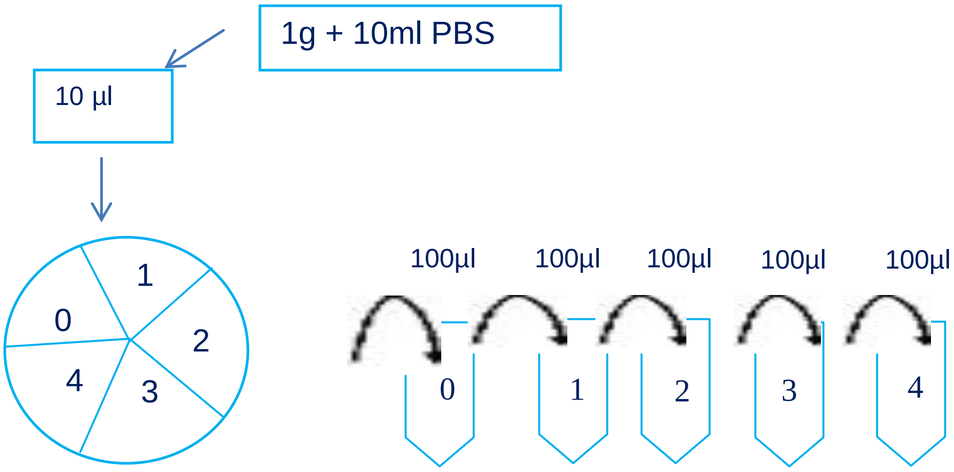


Fig. 1. Scheme for determining CFU/ml of isolated microorganism in foods/plants samples.

Quantitative analysis with specific enriched medium

The third part of the sample was cultivated in enrichment media for periods of24, 48 and72 hours in micro-aerophilic conditionsat room temperature, 37 ^{0}C and 28 ^{0}C.

Algorithm for the different bacteria identification, stage 1: preliminary identification, routine methods (unified and optimized in LMMMI, UzhNU)

Enterobacteriaceae

Endo agar, MacConkey, URI-select medium. Cultivate for 37 ^{0}C in thermostat for 18-24 hours. Microscopy: gram-negative, non-spore forming rods. For identification follow **Instruction 1**, below, and/or use ENTEROtest 24.

Staphylococcus

Nutrient Agar, Yolk-Salt Agar, URI-select medium. Cultivate the inoculated Petri dishes at the 37 ^{0}C in thermostat for 18-24 hours. Microscopy: gram-positive typical shape coccus, zones of lecithin lyses are observed, pigmented. Then follow **Instruction 2**.

Streptococcus

Blood agar, Nutrient Agar, URI-select medium. Cultivate the inoculated Petri dishes at the 37 ^{0}C in thermostat for 18-24 hours. Microscopy: gram-positive typical shape coccus, visible hemolysis. Then follow **Instruction 2**.

Lactic acid bacteria

Lactobacilli MRS Agar. Cultivate inoculated media under anaerobic condition during 48-72 hours. Keep for the repeated testing the 100 µl of the native material in MRS broth. Microscopy and routine biochemistry: in order to distinguish *Lactobacillus* (non-spore Gram positive, catalase negative rods) from *Bacillus spp.*Then follow **Instruction 3**.

Bifidobacterium

Blaurock medium.Cultivate the sample in anaerobic condition during 48 hours. Take the colony for microscopy in the case of visible growing (the culture growth usually in the form of comets, rings, grains, nails). Microscopy: Gram-positive rods, elicited, with branching on one or two sides, appear in V-form, dumbbell-shaped, claviform-beaded.

Enterococcus

Enterococcus selective Agar**.**Cultivate inoculated media for the 18-24 hours at 37ºC. Microscopy and routine biochemistry: in order to distinguish enterococcus (catalase negative) from streptococcus (positive in catalase and possessing different microscopy). *Enterococcus faecalis* is better stained dark-red-violet color (in this selected medium), whilst *Enterococcus faecium* is dull-colorless or slightlyrose-violet in colonies. Additional serological tests are usually required.

Anaerobes

Schaedler Agar, Wilkins-Chalgren Agar, Columbia Agar (with blood and vitamin K), liquid medium for anaerobic bacteria (thioglycol medium, others). The following results were obtained from type cultures after anaerobic incubation at a temperature of 35 ± 2 ^{0}C and observed after 24 – 48 hours. At the end of these periods, the colonies were cultivated in aerobic conditions to distinguish facultative anaerobic organisms from obligate anaerobes. Additionally inoculate and incubate the liquid media.

Bacteroides

Hennel medium. Ananaerobic incubation at a temperature of 35 ± 2 ^{0}C for 72-96 hours was performed. The colonies were calculated, re-incubated on the Nutrient Agar and incubated to the thermostat to growth in aerobic condition. Microscopy: Gram-negative, non-spore forming, poly-morphologically shaped, anaerobes.

Clostridium

Clostridium Agar / Wilson-Blair agar.Pour the 100 µl of suspension into the melted column of agar-based medium. It was additionally covered with sterile vaseline oil and incubated at 37 ^{0}C. The tubes were checked after48 hours for the presence of dark (black) colonies deep in the agar; calculate exceptionally out zone of aerobiosis, with visible gas generation and explosion of the medium. In the case when Clostridium species were not present in the sample the media will be bright yellow in colour.

Candida
Sabouraud medium, the Starch and Rice Agars.Yeast fungal isolation can be easily performed on the Sabouraud medium laced with chloramphenicol (200 mg/ml). After inoculation of the native material, typical white dull-raised colonies appear after 2-3 days. These colonies were selected and inoculated on the starch and rice agar using thestroke method to reveal thefilaments creation. Investigate the colonies using a hand-glass and microscope.

Micro fungi
Sabouraud medium, the Starch and Rice Agars.Cultivate the inoculated samples at 22-26 ^{0}C 4-5 days. Investigate by microscopy of the agar blocks and the sex apparatus of fungi.

Micro fungi
Chapek-Dox medium. Store the poured plates in an inverted position and inoculate using a needle or wire, with the plate inverted in order to avoid scattering stray fungal spores over the surface of the medium, incubate for 1-2 weeks at 25^{0}C.

The adapted (optimised) methodology was applied in other experiments:

1) **From1st June, 2010 until 30th of September, 2010**: the unified methodology for isolation and identification of key microorganisms was disseminated to all project partners. All thefood samples (homemade *vs*. city cooked and prepared in restaurants) and their major plant components (obtained from different locations and sources: in yards, city- and street markets) were tested; the first part of theprioritised foods samples provided by both Ukrainian teams (ONAFT and UzhNU), Georgian (ELKANA) and Russian (MSUFT) have been evaluated;

2) **From 1st June, 2011 until November, 2011:**a) the second part of the prioritised traditional foods (because of their seasonality) were provided by Georgian (ELKANA), Russian (MSUFT) and Turkish(EDITEPE) partners; b) three visits were made by the UzhNU team to MSUFT (Russia), UFT (Bulgaria) and EDITEPE (Turkey) to collect the samples and their corresponding plant components. All the samples were then microbiologically investigated. All isolated microorganisms were identified as described above;

3) **From 1st April, 2012 until August, 2012**: all the remainingsamples were investigated bacteriologically. Three more visits are needed to complete the analyses of all the plants ingredients and foods: in May – to ASE (Romania), in June – to YEDITEPE (Turkey) and in July – August to ELKANA (Georgia).

4) The selected plants, berries and prioritised food extracts were analyzed by routine microbiological tests complemented with rapid detection tests. Semi- and automatic systems for bacterial/fungi isolates identification, quantitative and qualitative estimation of the levels of their microbial contamination were used.

5) Statistically-analysed data are presented in this report.

Fig. 2.a.Instruction 1: Routine pre-identification of gram-negative rods based on their biochemical properties

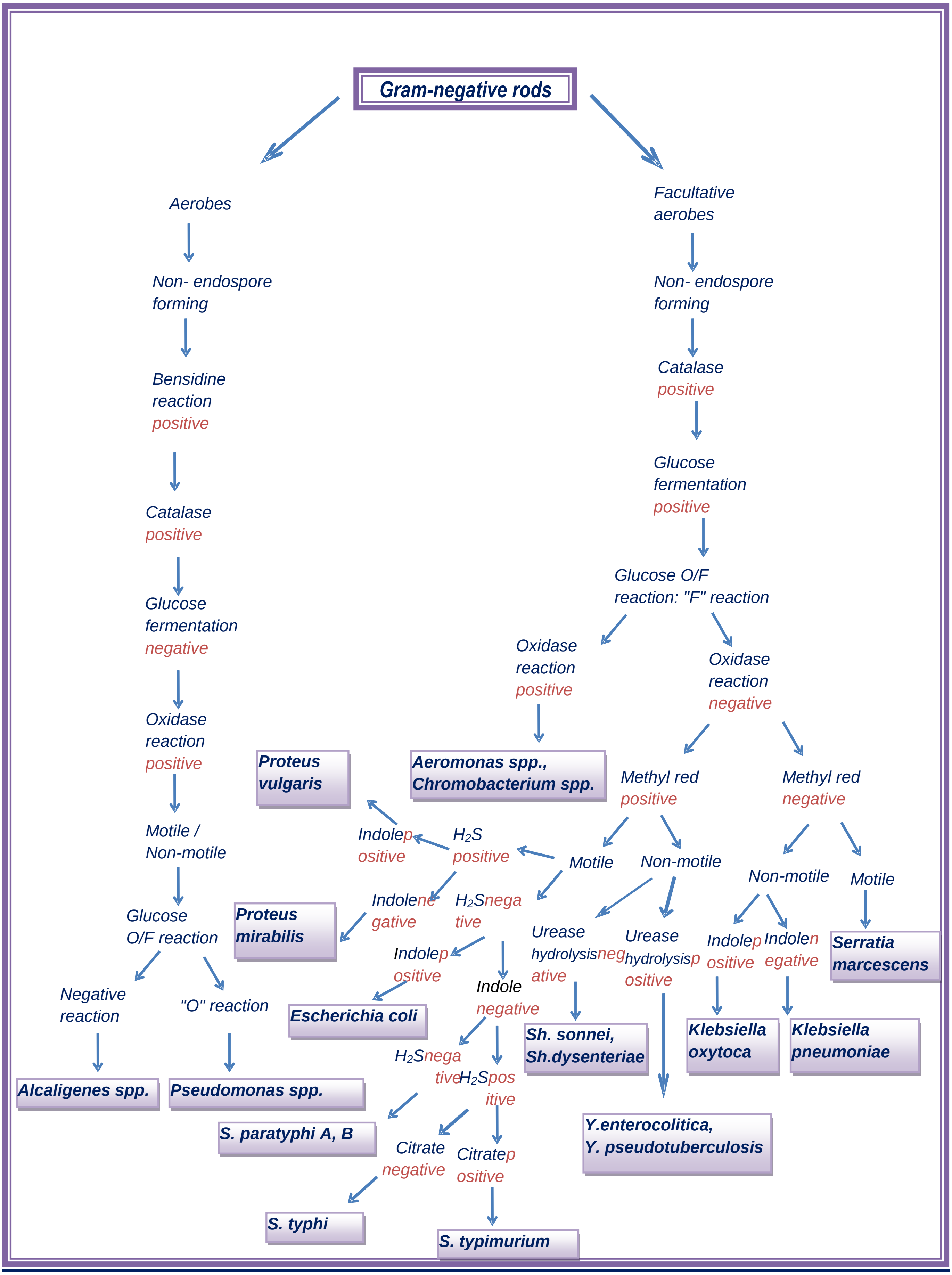


Fig. 2.b.Instruction 2: Routine pre-identification of Gram-positive coccus based on their biochemical properties

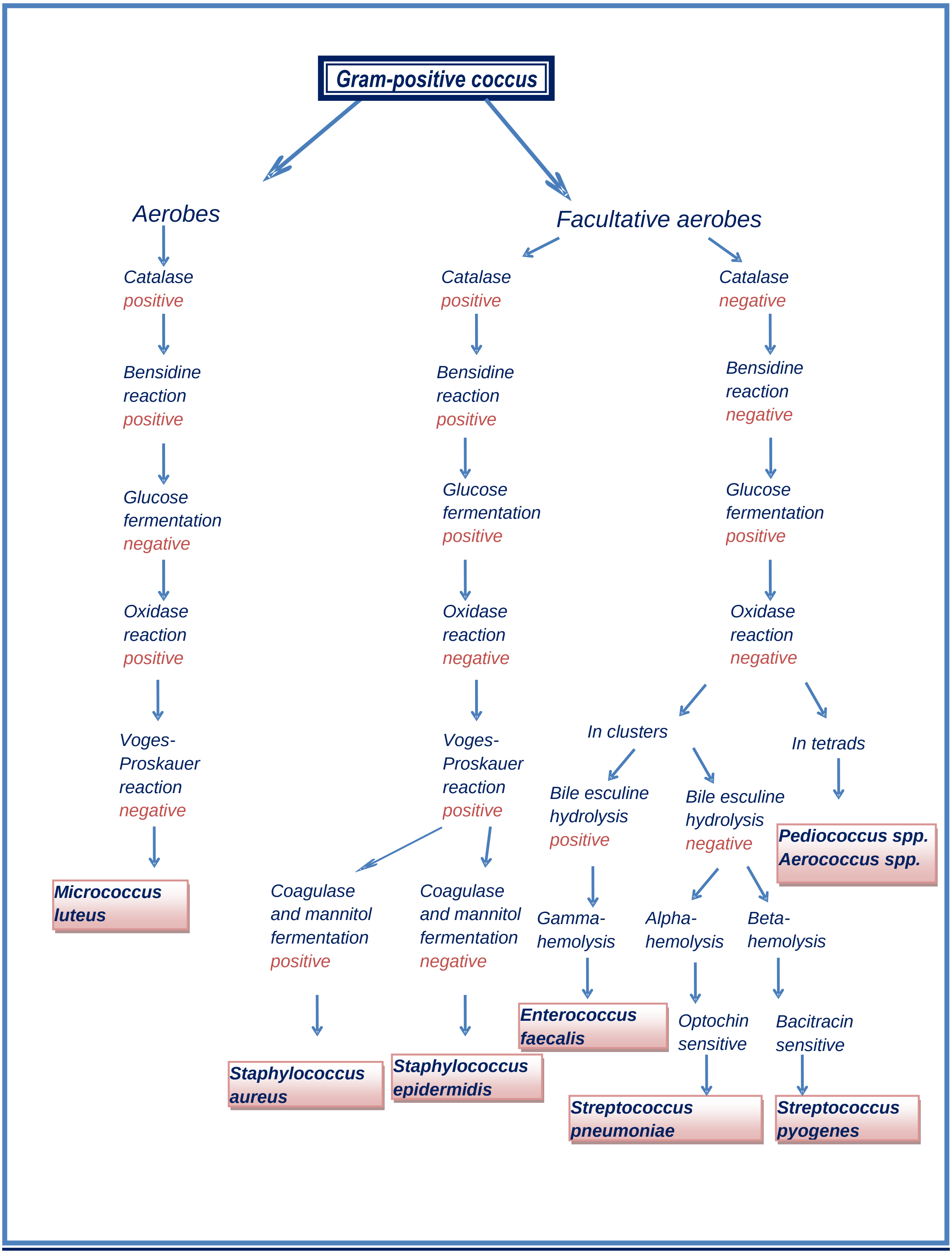
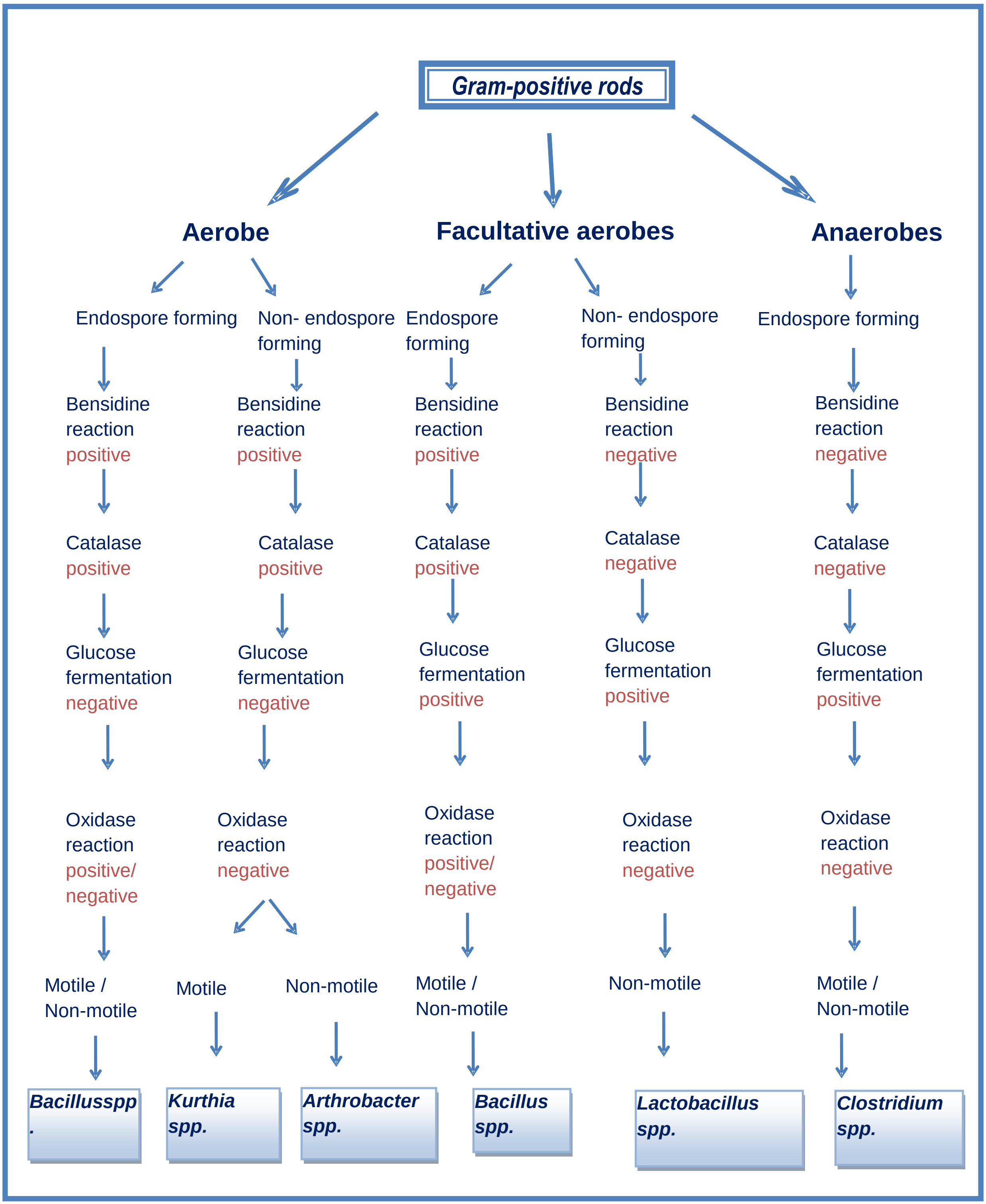


Fig. 2.c. Instruction 3:Routine pre-identification of Gram-positive rods based on their biochemical properties



In a few cases MALDI and PCR techniques were usedto distinguish strains belonged to *Enterococcus faecalis*or*Enterococcus faecium* species, and to confirm identification of a small number ofbacterium of saprophytic/epiphyticorigin – *Pantoea agglomerans*, *Eikenella corrodens*, *Enterobacter aerogenes* (sakazaki) and species belong to *Corynebacterium* and *Bacillus* genera.

RESULTS

BULGARIA

The plant components and foods tested in Bulgaria (working visit of UzhNU, August, 2011) arepresented in **Table 4**.

All the samples were tested according to the Methodological Manual. The microorganisms had been collected by plating of separate plant components and ready-to-eat meals on general and selected media to provide the required cultivation conditions for the isolation of different systematic groups of microorganism.

Table 4. Bulgarian samples of foods and food ingredients selected for the microbiological analysis.

No	Plant component	No	Prioritised Foods
1	**Wheat**	1	**Sunflowers seeds**
2	**Barley**	2	Rhodopian dried beans
3	**Smilyan beans**	3	**Rose jam**
4	**Onion**	4	**Tahan halva**
5	**Carrot**	5	**Tikvenik (pumpkin pie)**
6	**Flour**	6	**Boza**
7	**Tomato**		
8	**Savoury**		
9	**Mint**		
10	**Hot pepper**		
11	**Rose petals**		
12	**Good King Henry roots**		
13	**Pumpkin**		
14	**Walnuts**		

All microbial isolates in each systematic group were identified and calculated, and the amount of the most important (targeted) key microorganisms separately evaluated: A – Lactobacilli/Bifidobacteria and Bacilli species as beneficial microbes; B1a - representatives of epiphytic microbiota, Gram positive cocci, including Enterococcus, Staphylococcus and Streptococcus genera, and anaerobes; B1b – representatives of Enterobacteriaceae family and potentially pathogenic bacteria; B2 - food-borne pathogens (**Figs 3 - 9**).

Fig. 3 demonstrates that lactobacilli (*Lactobacillus mesenteroides*) were isolated only from hot pepper (in amount of $4.5 \cdot 10^4$ CFU/ml) – the sample collected from street market. The ability of this microorganism to cause the spoilage of fresh cut vegetables has recently been reported[24]. Ready-to-eat meals are shown in **Fig. 4** – boza and Tahini halva (tahan halva) were the source of different species of lactobacilli: *L. fermentium*, *L. delbrueckii, L. plantarum, L. acidophilus –for boza and Lactobacillus rhamnosus*–for halva. All microorganisms isolated from boza during its processing are described in **D2.5b**.Other bacteria belonging to the beneficial group are presented in **Fig. 5** and belong mainly to *Lactobacillus*, *Streptococcus* and *Bacillus* Genera (**Fig. 5**).

The main group of microorganisms isolated from Bulgarian plant samplesis enterobacteria. The plants, and especially their roots, were mainly contaminated as expected by a variety of representatives of the Enterobacteriaceae family, including the *Eshcherichia coli*, both: lactose-positive and lactose-negative strains, *Providencia alcalifaciens, Klebsiella pneumoniae, K. oxytoca* and*K. ozaenae*, *Pantoea agglomerans*, *Serratia marcescens*, *Enterobacter cloacae* (**Figs. 3, 6, 7**).

*Salmonella enterica*and *Shigella* species were not found, but*Salmonella enteritidis*has been identified in Good King Henry roots.Distribution of key representatives of Enterobacteriaceae species, isolated from all the Bulgarian samples,is shown in**Figs. 6–9**.

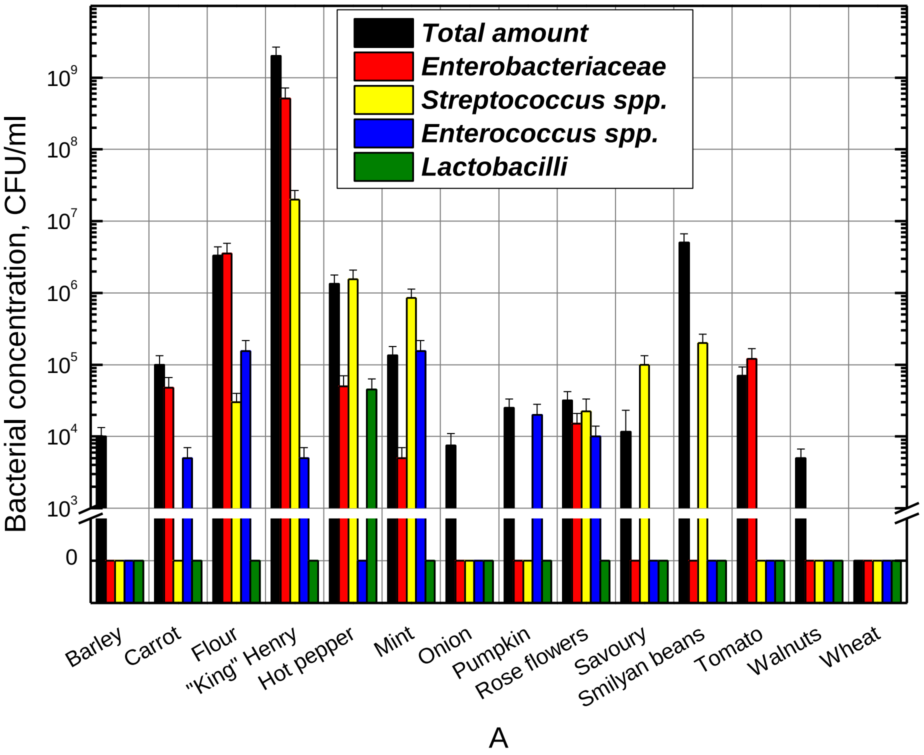


Fig. 3. The dominantgroups of bacteria isolated from the major plant components of prioritised Bulgarian foods.

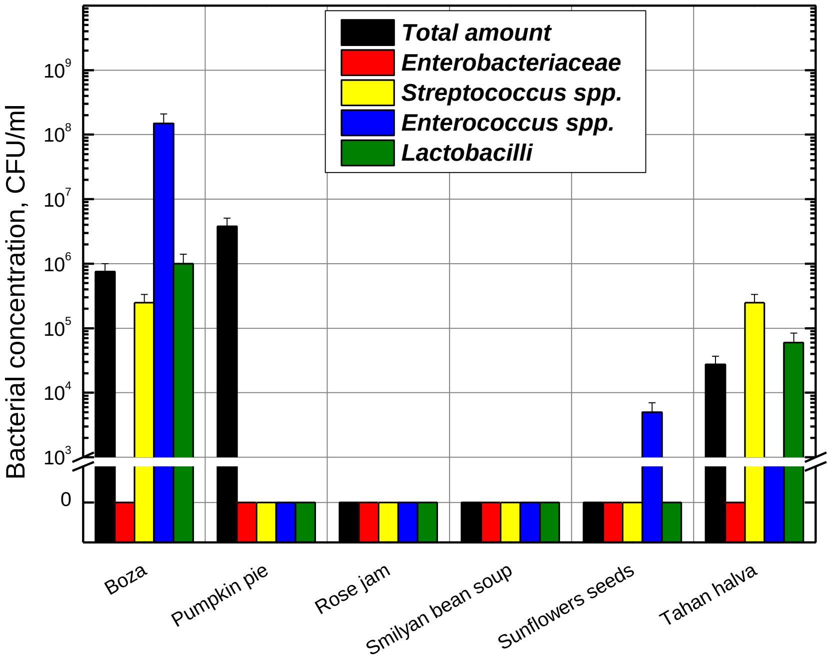


Fig. 4. The dominant bacteria isolated from Bulgarian traditional foods.

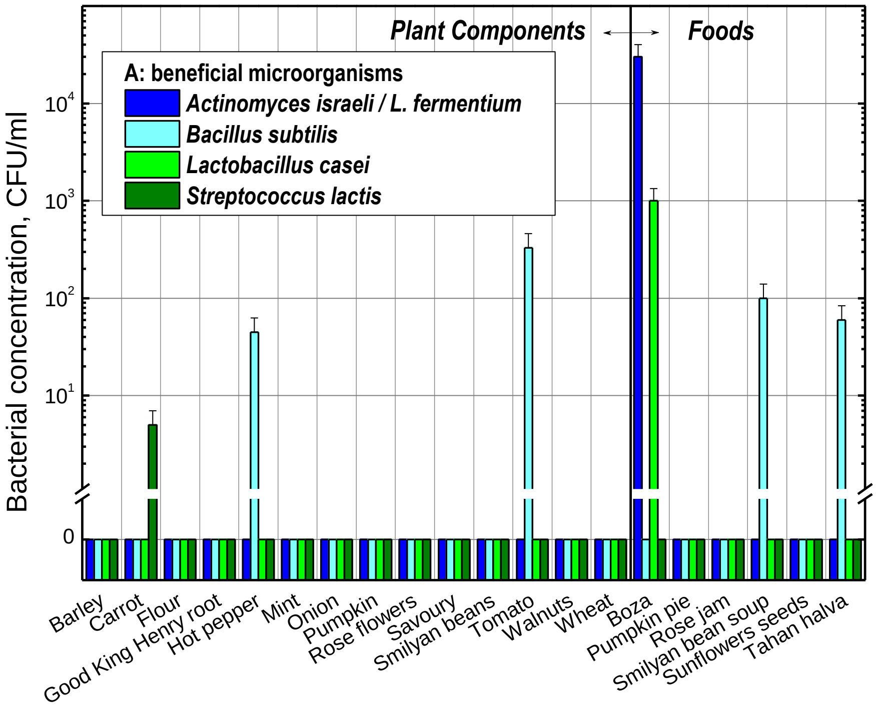


Fig. 5.Beneficial bacteria isolated from plant components and Bulgarian traditional foods.

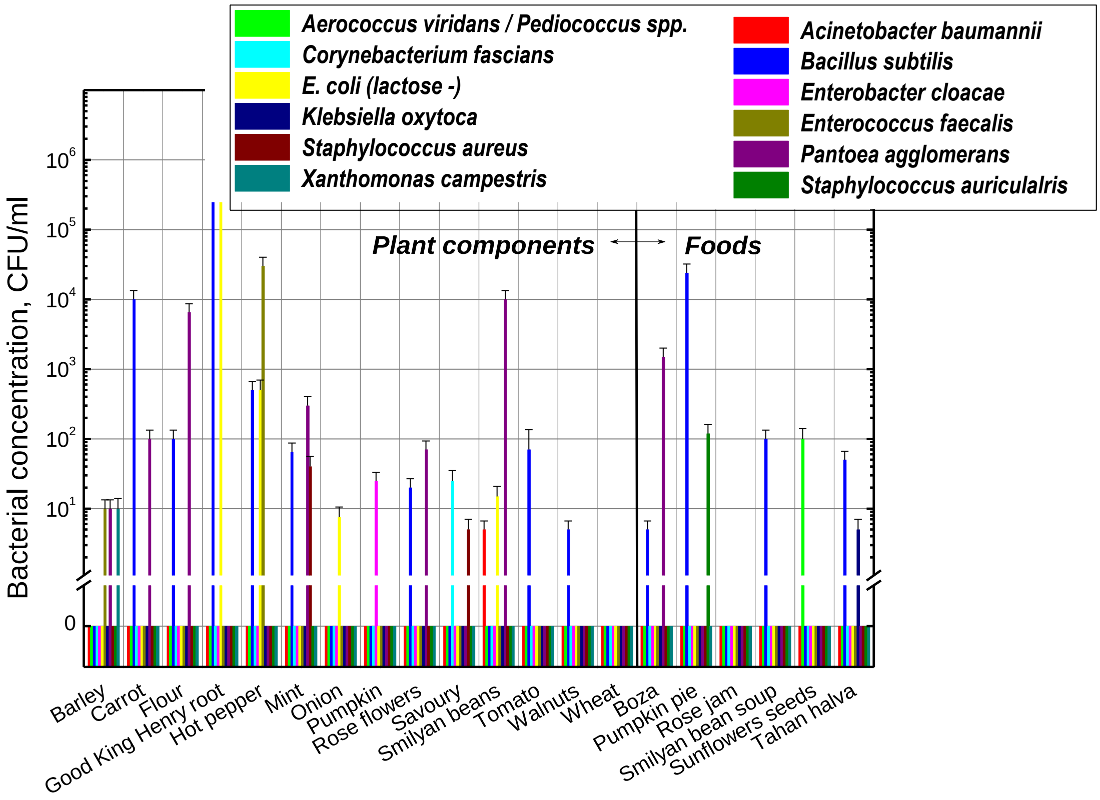


Fig. 6. B1 group(a and b) microorganisms isolated from Bulgarian samples (saprophytic microorganisms, contaminants of environmental origin: epiphytic microbiota and contaminants of human origin).

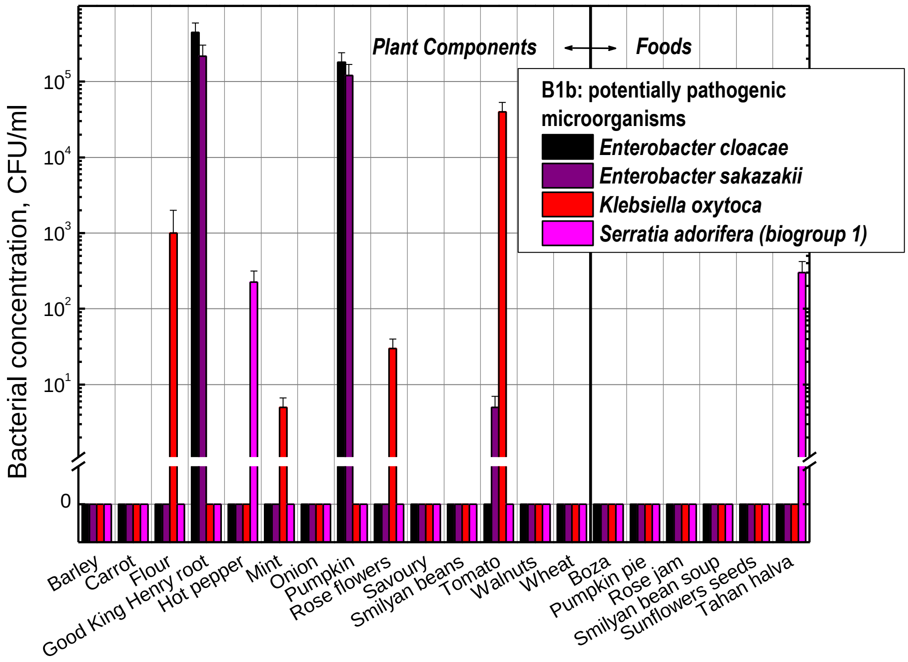


Fig. 7. Gram-negative (potentially pathogenic bacteria of human origin, B1, b group) microorganisms isolated from the plant components and from prioritized Bulgarian foods.

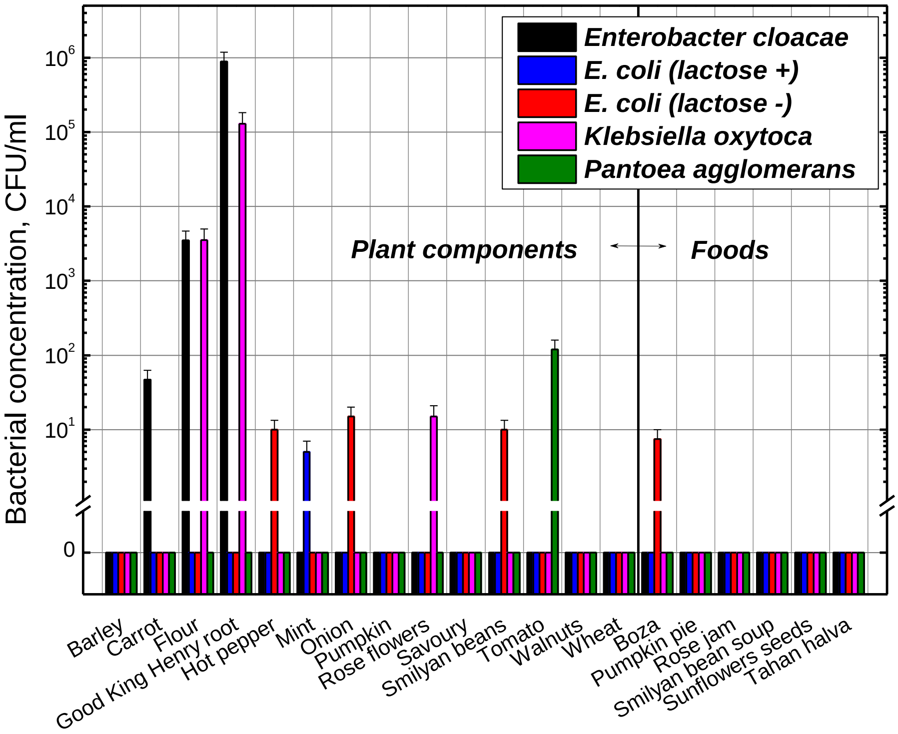


Fig. 8.Gram-negative potential pathogens (group B1, b) detected in plant components and in prioritized Bulgarian foods.

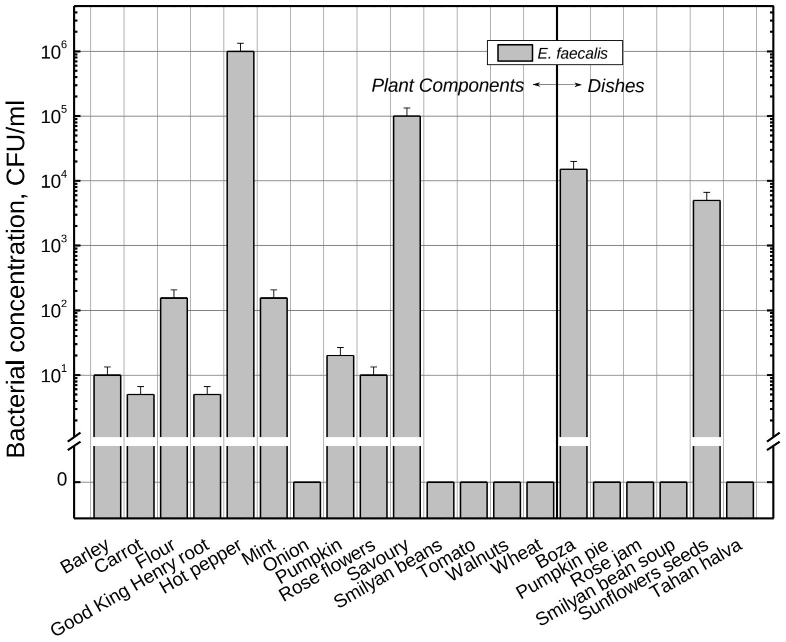


Fig. 9.Frequency of isolation*of Enterococcus faecalis* from all the tested Bulgarian samples.

Among all targeted groups of detected microorganisms – A, B1 (a and b) and B2 – the epiphytic and potentially pathogenic bacteria (B1) are dominant in Bulgarian samples and include the following species: *Xantomonas campestris*(typical plant bacteria – B1a), and *Aerococcus viridans*, *Pedicococcus cerevisae* (*Pediococcus damnosus, Pedicococcus pentosaceus*), *Corynebacterium fascians*, *Escherichia coli* (lactose + and lactose –), *Klebsiella oxytoca*, *K. ozaenae*, *K. pneumoniae*,*Acinetobacter baumannii*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Pantoea agglomerans*, *Staphylococcus auriculalris*, *S. aureus*, which are, including the last microbial strain, in theB1b group.Their role in food processing and correspondingly in food safety/food quality needs to be further discussed andthe levels carefully determined.

GEORGIA

All prioritised food samples and their major plant components (**Table 5**) were collected during the working visit to Tbilisi and also by the Georgian team (ELKANA),according to their seasonality;they were then tested according to the chosen methodology (manual) in 2010, 2011 and 2012.The biodiversity of isolated bacterial species from selected Georgian foods is shown in **Fig. 10**. The beneficial and other key-microorganisms of major plant ingredients: contaminants of environmental and human origin, as well as,food-borne pathogens were examined *via* quantitative assay and the results are presented on **Figs. 11 – 13**.

Table 5.Georgian samples of collected foods,plant food ingredients selected for the microbiological analysis and seasonality.

No	Plant component	No	Prioritised Foods	Availability
1	White grapes	1	Churchkhela	September-October
2	Nettle	2	Nettle with walnut	Early Spring
3	Onion	3	Nettle with walnut	June, September
4	Walnut	4	Nettle with walnut	September
5	Wheat flour (Tsiteli Doli variety)	5	Tsiteli doli bread with makhobeli	July
6	Makhobeli* seeds	6	Tsiteli doli bread with makhobeli	July
7	Wild plum		Wild plum sauce	August-September
8	Coriander (fresh)		Wild plum sauce	April-October
9	Fennel (whole plant with seeds and stems)		Wild plum sauce	April-October
10	Pennyroyal		Wild plum sauce	April-October

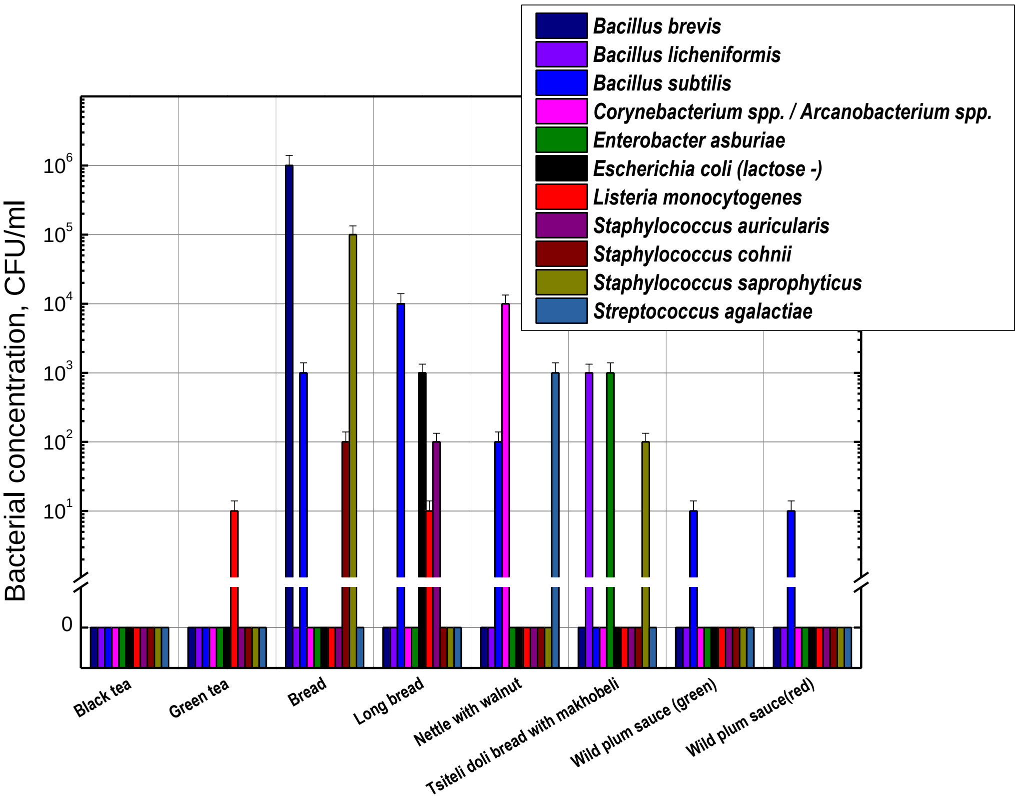


Fig. 10.Bacteria/microorganisms isolated from tested Georgian foods.

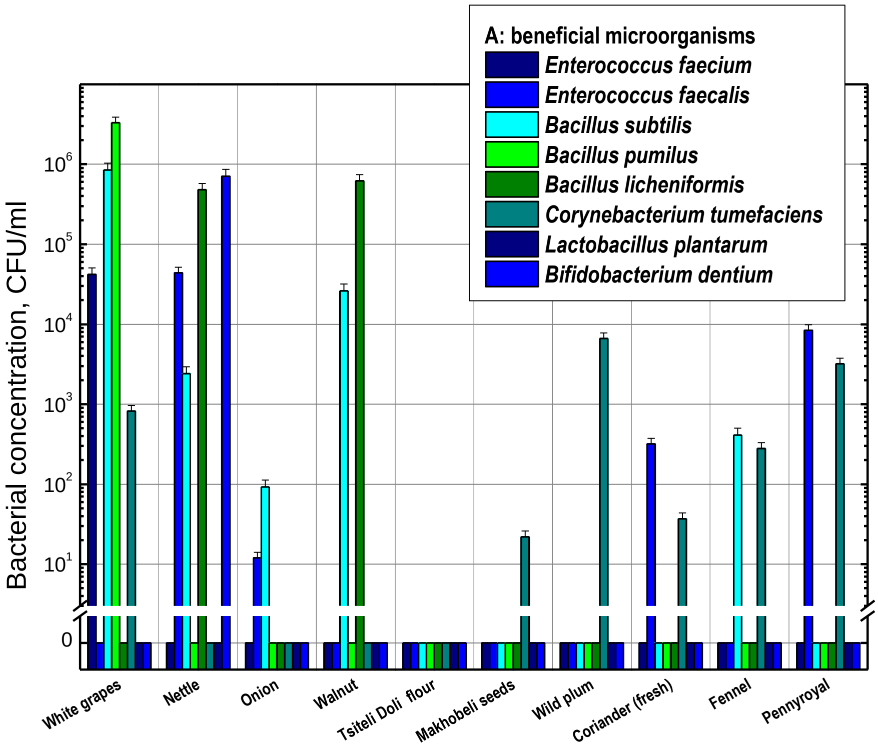


Fig. 11. Beneficial microorganisms in major plant components of Georgian foods: group A.

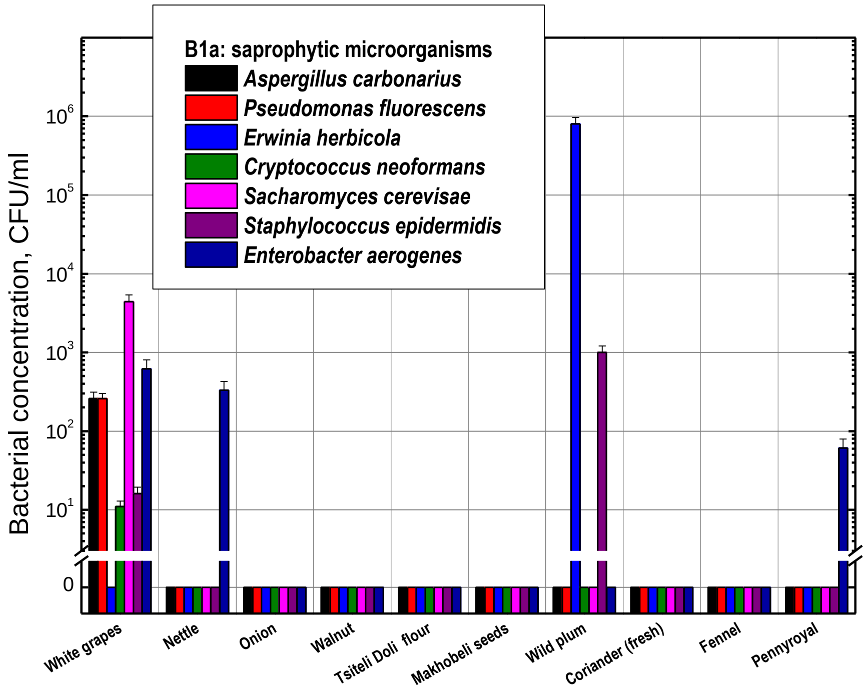


Fig. 12.Key microorganisms isolated from the major plant components of Georgian foods: B1a group – environmental saprophytic bacteria.

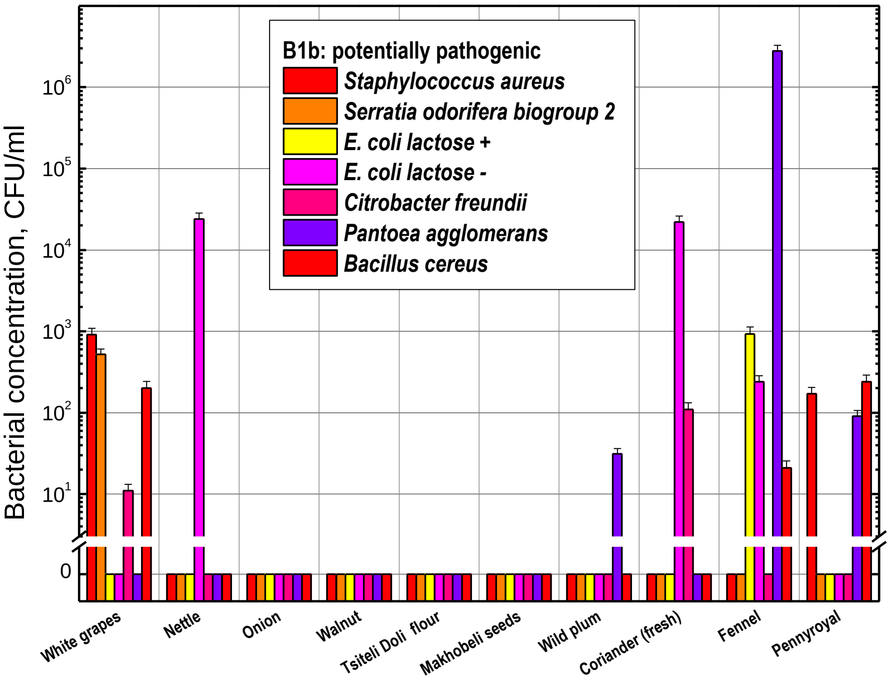


Fig. 13.Key microorganisms isolated from the major plant components of Georgian foods: B1b group – human originated contaminants.

Beneficial bacteria (group A) in Georgian foods are mainly *Bacillus* species (*brevis, subtilis* and *licheniformis*), some representatives of *Enterococcus spp.* and *Corynebacterium spp.* These were all found in types of traditional bread, plum sauce andnettles with walnuts; other beneficial microbes from Lactobacilli and Bifidobacterium genera were isolated only from plant ingredients and these typical beneficial bacteria are not present in large amounts compared with those reported above (**Fig. 10, 11**).

Environmental microbial contaminants (group B1a, **Fig. 10, 12**) of plant components of selected Georgian foods are distributed between 7 main groups of microorganisms: 4 bacterial, “classic” epiphytic (*Erwinia* and *Pseudomonas*) and saprophytic representatives (*Staphylococcus* and *Enterobacter)* and 3 genera of microscopic fungi: *Sacharomyces*, *Aspergillus* and *Cryptococcus*. From 10 samples examined, positive results were obtained in 4 cases only (grape, nettle, plum, and local mint - pennyroyal, 2 of them were contaminated with only 1 specie (nettle and mint), 1 – with two species (plum) and 1 – with all the mentioned species except *Erwinia herbicola* (white grape).

B1b group (**Fig. 10, 13**) – potentially pathogenic microorganisms in the microbiologically-assessed Georgian foods and plant samples include the following dominating species*: Pantoae agglomerans* (10^2– 10^6 CFU/ml), three contaminated samples); *E. coli* (lactose positive and negative, 10^2 – 10^4 CFU/ml), three contaminated samples; *Serratia odorifera* (10^3 CFU/ml), one contaminated sample and *Citrobacter freundii* (10^1 – 10^2 CFU/ml), two contaminated samples.

Staphylococcus aureus (10^1 – 10^2 CFU/ml), two plant samples; *Bacillus cereus* (10^2 CFU/ml), three plant samples and *Listeria monocytogenes* (10^1 CFU/ml) in two food samples from the B2 group of key microorganisms presenting food-borne pathogens (**Fig. 10 and 13**).

ROMANIA

During our working visit to Bucharest (May, 2012) the prioritised foods had been cooked with the assistance of Romanian partner (ASE) in a typical familyliving in Ploiesti;whilst the major plant components were collected from local street and city markets.The seasonality of prioritised Romanian foods and its ingredients availability are presented in **Table 6**. Dominant groups of key microorganisms isolated from selected Romanian foods *vs* microbial strains found in their plant ingredients are shown in **Fig. 14 – 17**.

Table 6.Romanian samples of foods and plant food ingredients selected for the microbiological analysis and seasonality.

No	Plant component	No	Prioritised Foods	Availability
1	Basil leaves	1	Herbal dish	July
2	Mint leaves	2	Herbal dish	July
3	Celery green and root	3	Nettle soup	October
4	Nettles of spontaneous vegetation	4	Nettle soup	10.04 – 30.04
5	Glazed rice	5	Nettle soup	All the year
6	Green leaves of laurel	6	Nettle soup	May - September
7	Plum fresh and dried	7	Plum jam	August – September
8	Flower of elderberry	8	Socata	End of May
9	Lemon	9	Socata	All year
10	Corn grits and flour	10	Cornmeal mush	All year
11	Carrot	11	Borsch	All year
12	Onion and green (spring) onion	12	Traditionalbread	All year
13	Parsley green and root			
14	Sage lives			
15	Black pepper			
16	Red pepper sweet			
17	Cherry leaves			
18	Cucumber			
19	Cabbage			
20	Garlic			
21	Beet			
22	Tomato			
23	Wheat flour			

Among the beneficial bacteria,the mainspecies were *Lactobacillus casei* found in nettle, carrots, celery roots and green, parsley roots, onion, cherry leaves and in ready-to-eat cornmeal mush (10^6 CFU/ml) and *Bifidobacterium longum*, isolated often from mint, nettle, parsley roots, cherry leaves, cabbage and from ready-to-eat meal borsch (10^4 CFU/ml) prepared from these ingredients –(**Fig. 14**). *Bacillus subtilis* found in green and old onion, dried plums, black and red sweet peppers, garlic, beet and in herbal dishes (10^4 CFU/ml) and traditional bread. *B. licheniformis* was present in significant amounts in dill (10^7 CFU/ml), parsley roots (up to the 10^8 CFU/ml) and beet (10^6 CFU/ml) but was not found in foods. *Candida famata* (synonym *Torulopsis candida*) was occasionally isolated in low amounts (10^1 CFU/ml) and is listed here because of its significant role in the fermentation of Romanian “Socata” (see details in **D2.5b**).

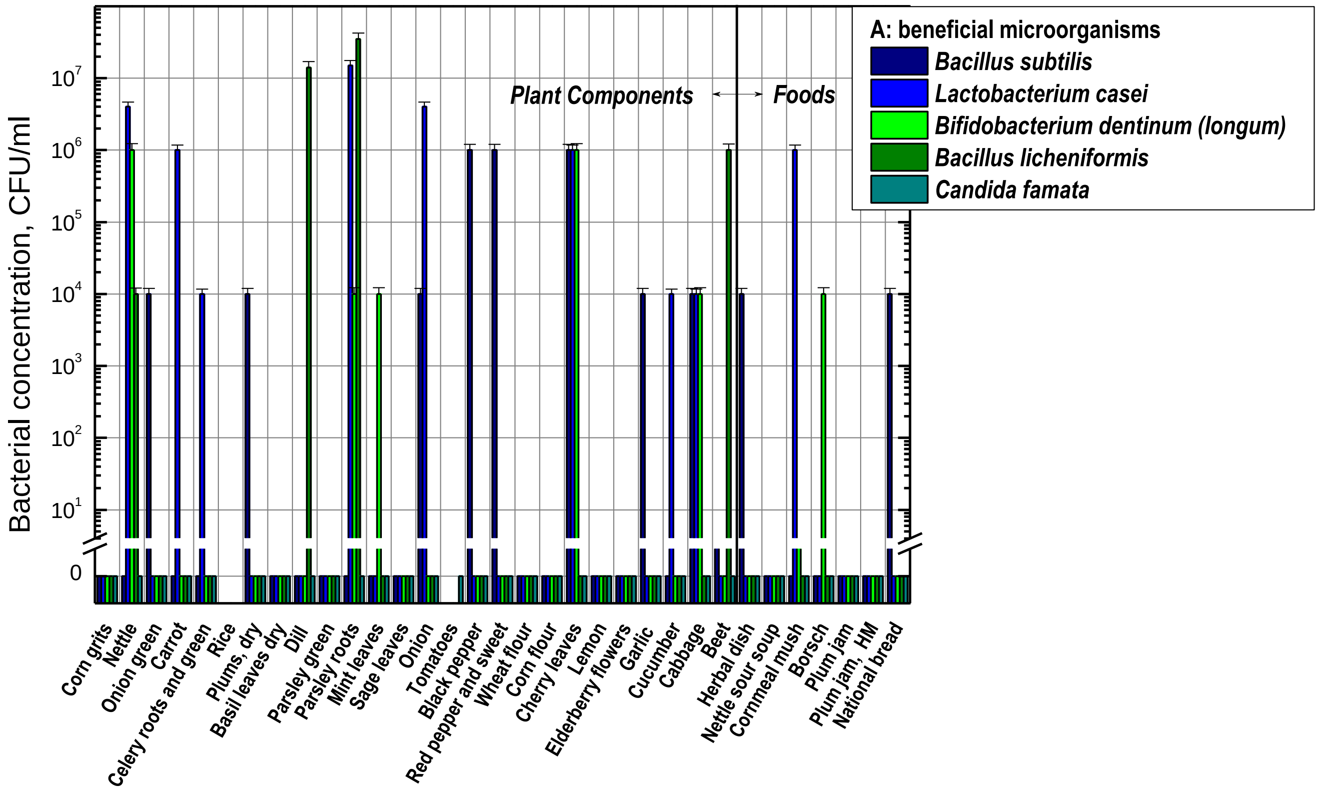


Fig. 14. Beneficial microorganisms in major plant components of Romanian foods: group A.

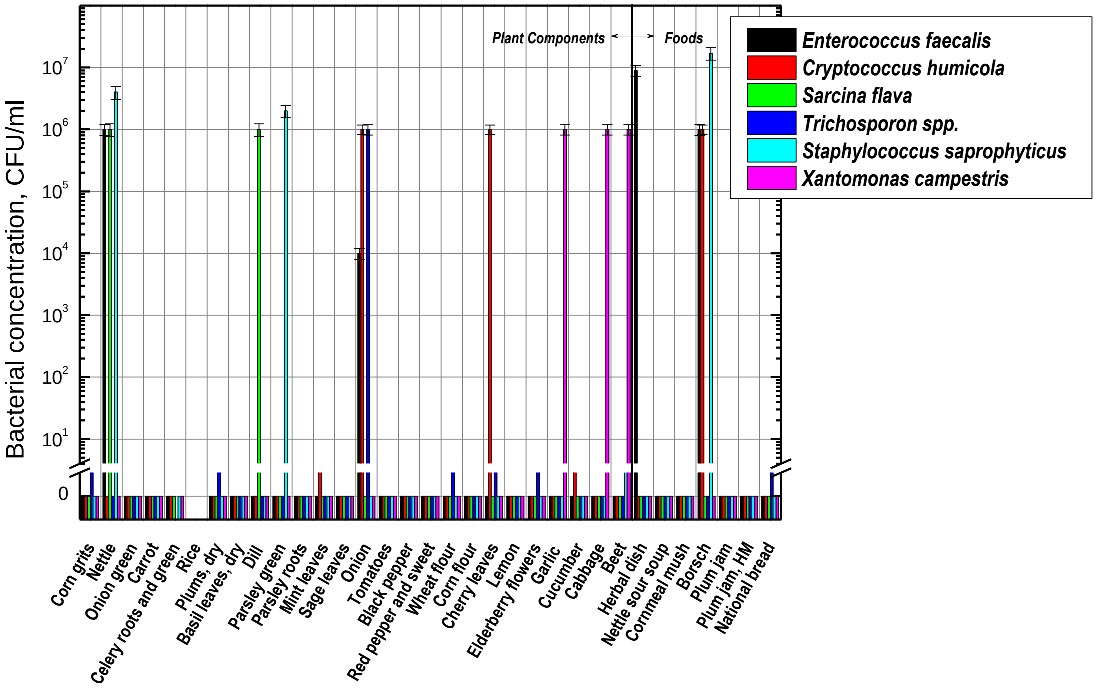


Fig. 15.Key microorganisms isolated from the major plant components of Romanian foods: B1a group – environmental saprophytic bacteria.

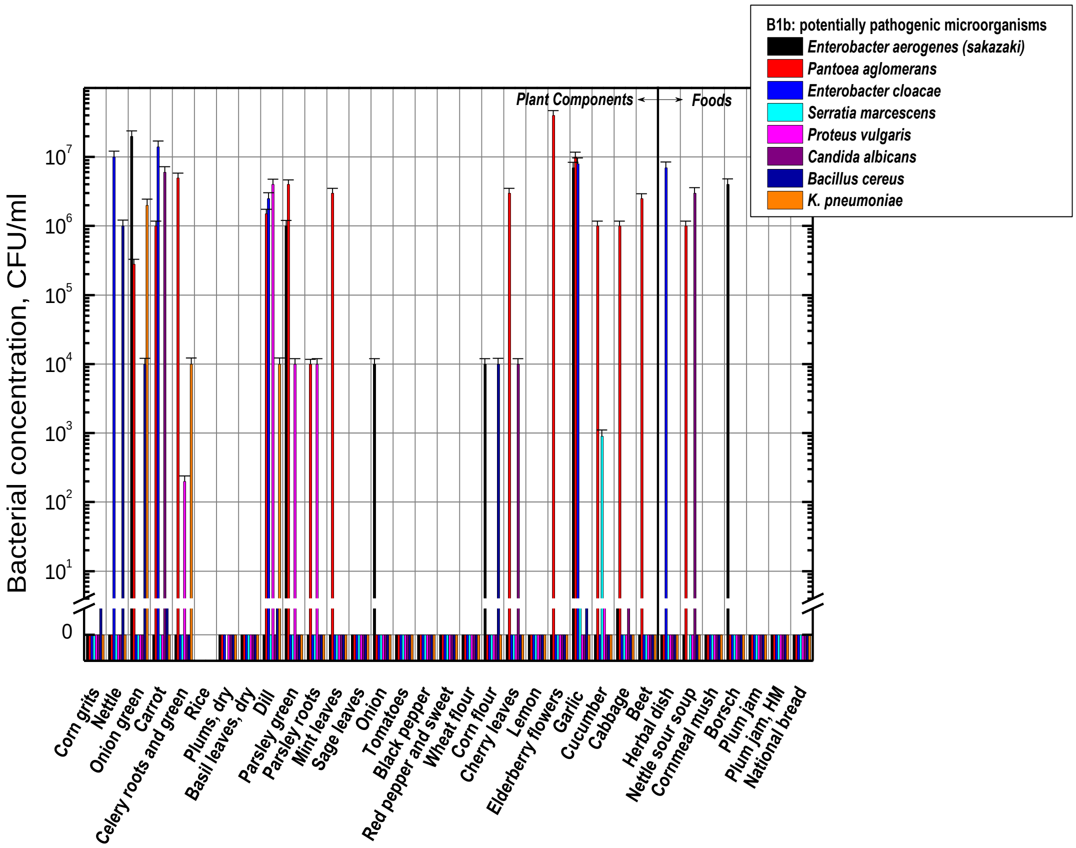


Fig. 16.Key microorganisms isolated from the major plant components of Romanian foods: B1b and B2 group – contaminants of human origin.

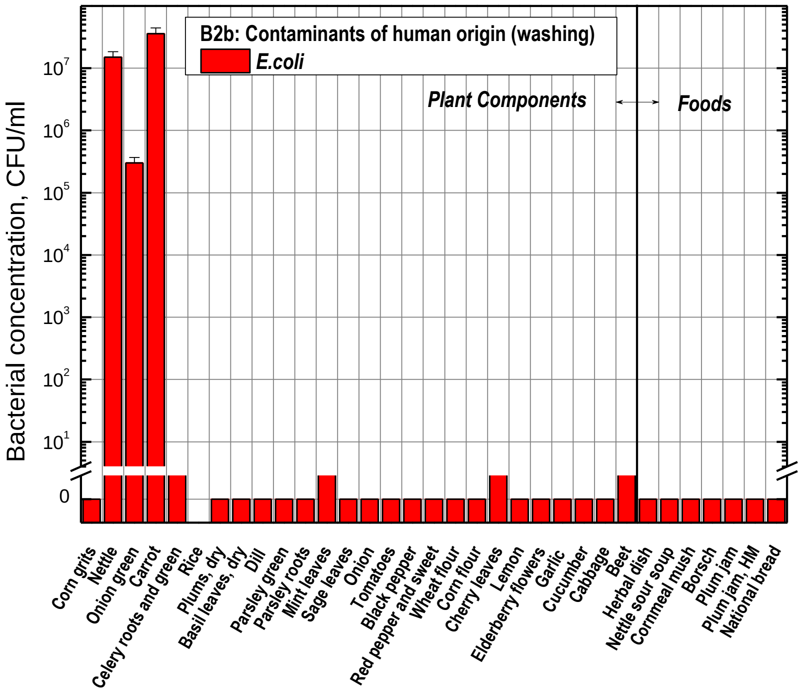


Fig. 17.Key microorganisms isolated from the major plant components of Romanian foods: B2b group – contaminants of human origin.

Fig. 15 demonstrates 6 dominating microbial genera of environmental contaminants (group B1a) – 4 bacterial: *Enterococcus* (*faecalis*), *Sarcina* (*flava*), *Staphylococcus* (*saprophyticus*) and *Xantomonas* (*campestris*) and 2 fungal genera: *Trichosporon* and *Cryptococcus*.

Both fungal species are reported as common environmental isolates;moreover, it has been reported that some species of Cryptococcus – C*ryptococcus humicola*can prevent lesions in “Golden Delicious” apples [25].

B1b group – human originated contaminants (**Fig. 16**) includes the following bacterial representatives in sufficient amounts:two species of *Enterobacter: cloacae*and *sakazaki*; *Pantoea agglomerans*, *Serratia marcescens*, *Proteus vulgaris* and *Klebsiella pneumoniae*. The only fungal representative was identified as *Candida albicans*. *B. cereus*which can hypothetically belong to B2 group – foodborne pathogen, but the isolated strain did not show any virulent properties *in vitro.*

Amongst contaminants of human origin, which had been involved in food contamination during the food preparation process, only *E. coli*was detected (**Fig. 17**).

RUSSIAN FEDERATION

All the samples were obtained from street city markets and compared with samples obtained from yards [farms]; foods were prepared in restaurants and were also homemade, and were checked in comparison with the same Ukrainian foodscollected correspondingly at home and in markets (kvass) or restaurants.

In **Table 7,** the major plant ingredients and prioritised foods analysed microbiologically are listed (**Figs 18 – 23**).

Table 7.Russian samplesof collected foodsand plant food ingredients selected for microbiological analysis.

No	Plant component	No	Prioritised Foods
1	Cucumber	1	Buckwheat porridge crumbly
2	Cabbage	2	Okroshka
3	Beet	3	Kvass
4	Garlic green	4	Watermelon Juice
5	Red/yellow pepper	5	Mustard oil
6	Onion green (spring)	6	
7	Sorrel	7	
8	Radish	8	
9	Dill	9	
10	Carrot	10	
11	Potato	11	
12	Buckwheat	12	
13	Turnip		
14	Juniper berries		
15	Raisins		

Foods

As shown in **Fig. 18,** the selected Russian traditional foods are a relevantly rich source of beneficial microorganisms and, similar to results from the other 6 prioritised foods from 6 BSAC countries,the biggest amount of which are found in fermented products. In case of Romanian herbal dish, cornmeal mush and borsch,as well as,in Russian vegetable okroshka and buckwheat porridge crumbly ready-to-eat meals, strains of *Lactobacillus, Bacillus* and *Bifidobacterium*species were identified. Buckwheat porridge crumbly, prepared at a household with a traditional recipe,contained Lactobacillus *plantarum* (10^2CFU/ml); inBuckwheat porridge crumbly prepared in the restaurant, *B. subtilis*was detected (10^1CFU/ml).

From okroshka made in Russian restaurants, from kefir – not from kvass –*L. galinarum*(10^4CFU/ml), *L. acidophilus*(10^5CFU/ml) and *B. subtilis*(10^2CFU/ml) were isolated, while in homemade okroshka – a prioritised traditional food within the project – *L. plantarum* (10^2CFU/ml) and *Actinomyces israelii* (10^1CFU/ml)were both identified.In Ukrainian vegetable okroshka – both made at home and prepared in the restaurant - the *A. israelii* was only isolated in some titers – 10^7CFU/ml.

In Russian homemade bread kvass, more than 10 microorganisms with beneficial properties were detected (see details for all the prioritised fermented products in **D 2.5b**).It hasbeen demonstrated that traditional homemade Russian bread kvass differs significantlyin their content of beneficial microorganisms from the other typesof industrial kvassesobtained in city markets inRussia and Ukraine: in Ukrainian sample of “Drevlyanskyy classic”kvass from rye bread, only 10^3 CFU/ml*Sacharomyces cerevisae*were detected,while in Russian Kvass bought in the street,the same microorganism was found to be present at10^2CFU/ml; Ukrainian kvass “Yarylo”based onrye, barley malt, corn flourand sugar,contains at least a small amount of *Lactobacillus plantarum*(up to 10^1); but Lvivs’kyy kvass (produced with sourdough of industrial strains of *S. cerevisae* and Lactobacilli) had only 10^1 CFU/mlof *S. cerevisae* but also contained lactose-fermented *E. coli*–10^2CFU/ml (**Figs 19 – 20**). Finally,watermelon juice prepared according to the original recipe had 10^1CFU/ml, whilstindustrial, bottled melon juice without the representative types of beneficial microorganisms, Russian mustard oil and flax oil from Georgia were all free of microorganisms.

The group of epiphytic bacteria **(Fig. 19)**which contaminate Russian traditional foods is mainlyformed from, in decreased order,*Saccharomyces cerevisae* (kvass and okroshka) 10^3 – 10^4 CFU/ml, *Pediococcus pentosaceus*(10^2CFU/ml) – homemade okroshka, *Enterococcus faecium*(10^2CFU/ml) – homemade original Russian okroshka and melon juice bottled from city market, *Enterococcus faecalis*(up to 10^6CFU/ml) – only from okroshka Ukrainian restaurant with kefir, *Rhysopus spp.* and *Pseudomonas fluorescens* – from Russian homemade melon juice ($10^1$and 10^2CFU/ml,correspondingly).

Groups B1b and B2 were represented by *Eubacterium tortuosum*– isolated from Ukrainian okroshka withkefir (up to 10^7 CFU/ml),lactose positive *E. coli* in Russian buckwheat porridge (10^1), Lvivskyy kvass (10^2), Russian original homemadeokroshka with kvass (10^2); lactose negative *E. coli* in Russian okroshka from restaurant 10^2 and homemade (in lower amount: 10^1), Russian homemade melon juice (10^2), *Pantoea agglomerans*in Russian okroshka from restaurant (10^1), *Streptococcus viridans*, *Streprococcus epedermidis*, *Proteus vulgaris* – in homemade Russian okroshka (10^2– 10^4), *Enterobacter cloacae*(10^8), *Serratia ficaria*(10^7) and *Staphylococcus aureus*(10^6) in homemade Ukrainian okroshka and, finally,*Enterobacter cloacae* and *Klebsiella oxytoca*(10^6) – in Ukrainian okroshka from a restaurant. Homemade Ukrainian okroshka differs significantly since in the recipe instead of kefir or kvass,uzvar from dog rose was used.

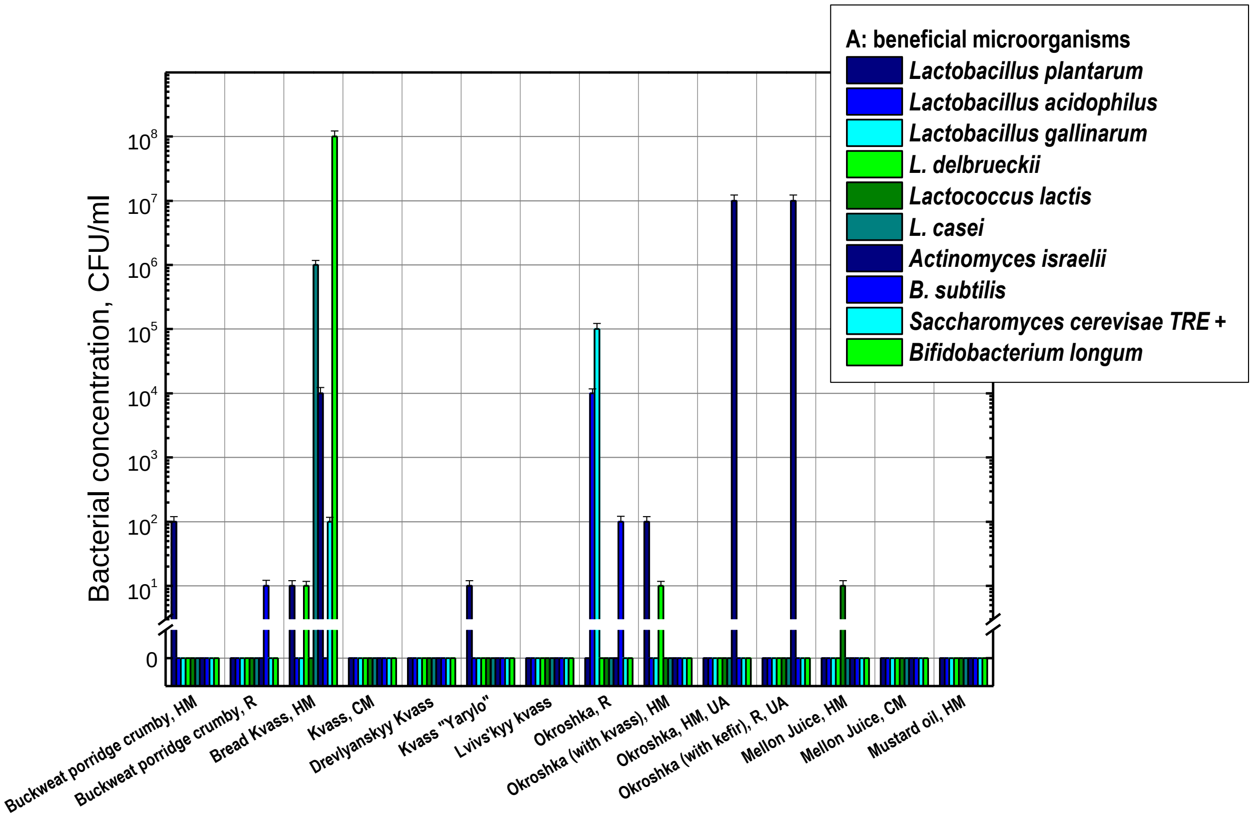


Fig. 18.Beneficial microorganisms inRussian Federation foods: group A.

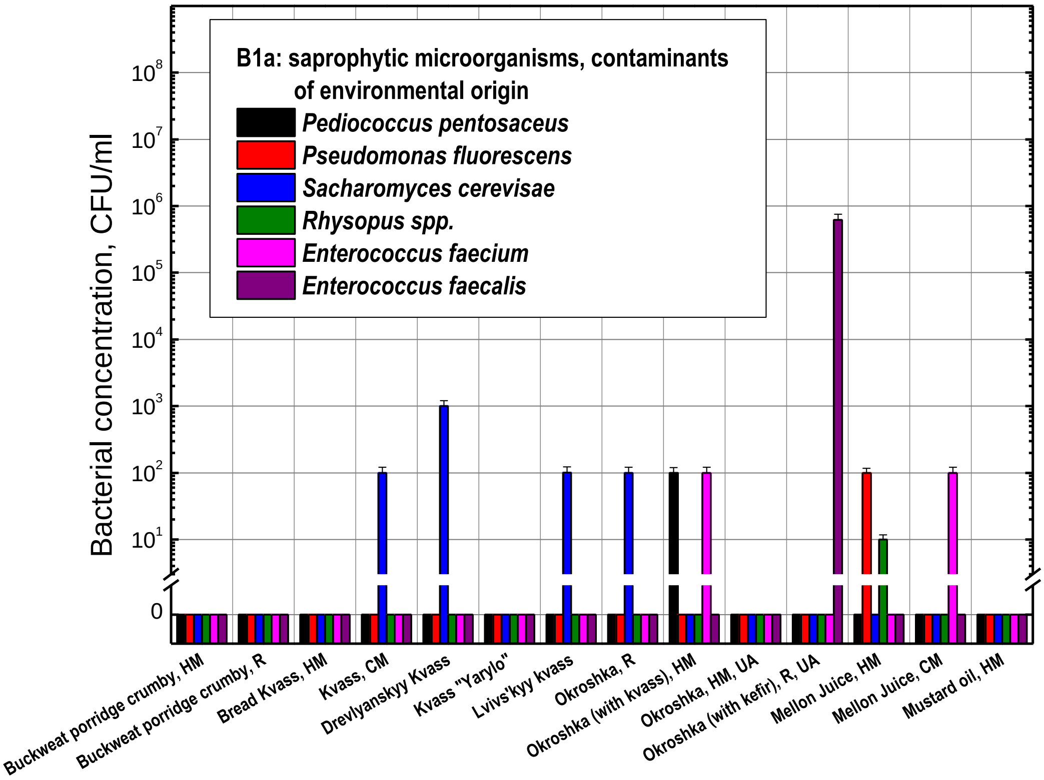


Fig. 19.Key microorganisms isolated from Russian Federation foods: B1a group – environmental saprophytic bacteria.

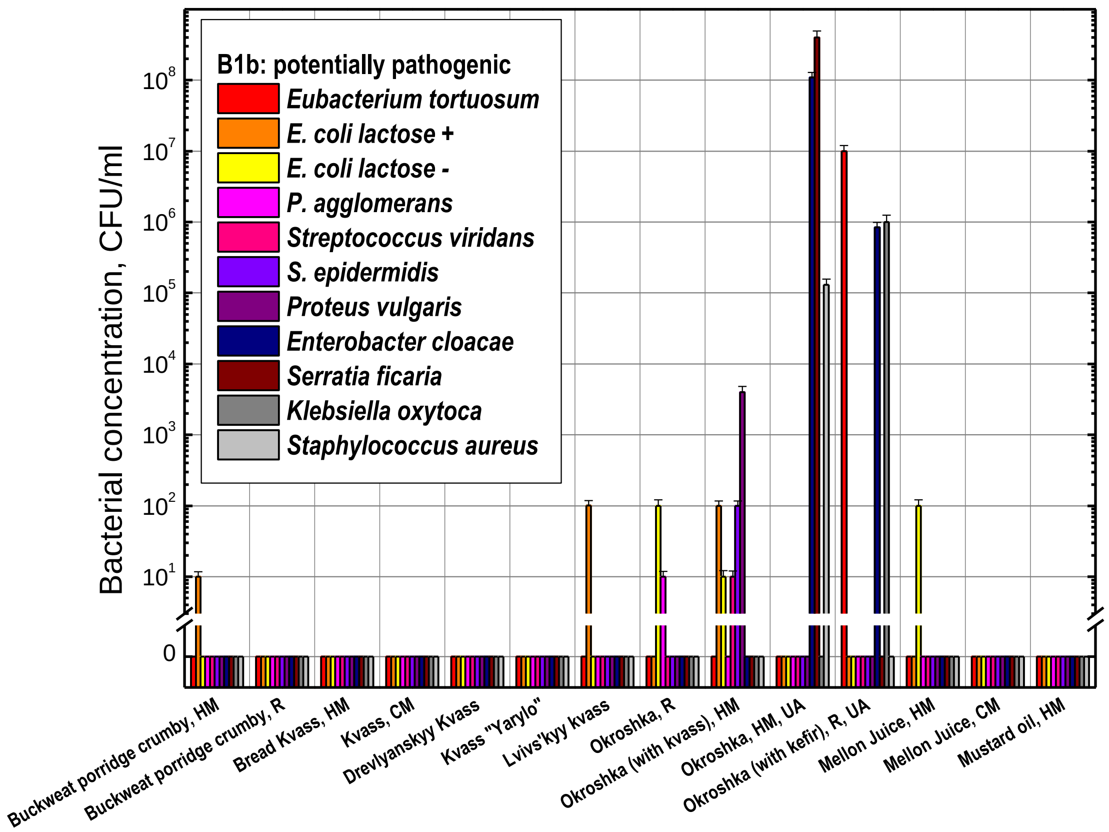


Fig. 20.Key microorganisms isolated from Russian Federation foods: B1b and B2 group –contaminants of human origin.

Plant ingredients

As shown in **Fig. 21,***Bacillus subtilis* is from the primary bacterial strain with potential beneficial properties amongst the examined plant ingredients – isolated cucumbers, tomatoes, juniper berries - all samples obtained from farms (specified as "yard" in the Figures), pepper and potatoes – from street markets (SM). *Enterococcus faecium* and *Lactobacillus catenaforme*were also common, being isolated from tomatoes (farm) and garlic (street market). Finally,*Actynomyces israelii*was detected in a sample of dill collected from a street market; no strains of *Corynebacterium* or *Bifidobacterium* genera were isolated.

Organisms of the B1a group (**Fig. 22**) in such samples were represented by *Enterococcus faecalis* – isolated from spring (green) onion, tomatoes, turnip from farms and carrot from a street market, *Staphylococcus epidermidis* – from raisins collected in farms, and *Streptococcus acidominimus* – also from turnip. No typical epiphytic bacteria from the Erwinia, Pseudomonas or Xanthomonas genera were found.

Enterobacter cloacae, E. coli, lactose-negative and *Citrobacter freundii* (parsley), *Staphylococcus aureus* (radish and dill), *Neisseria spp.* (onion green), *Candida albicans* (cucumber) and *Candida pelliculosa* (dill) are in the B1b and B2 groups of potentially dangerous microorganisms. *E. coli* lactose-positive organisms were not found in any of the plantsamples, including buckwheat from city market (CM) eventhoughit was detected in ready-to-eat meal homemade buckwheat porridge crumbly.

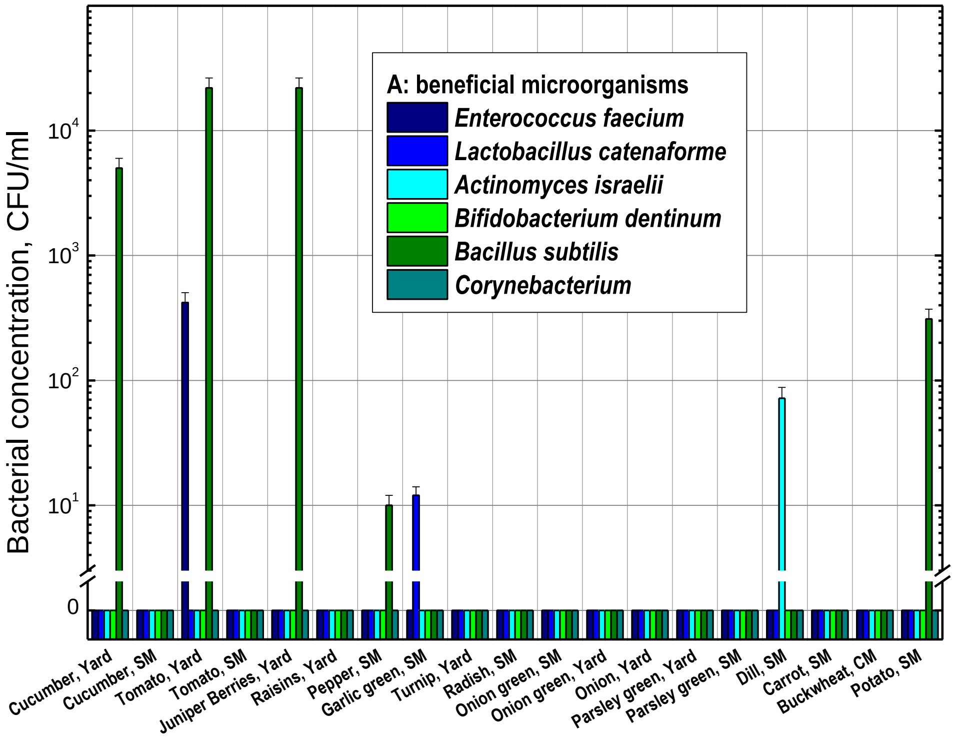


Fig. 21.Beneficial microorganisms in major plant components of Russian Federation foods: group A.

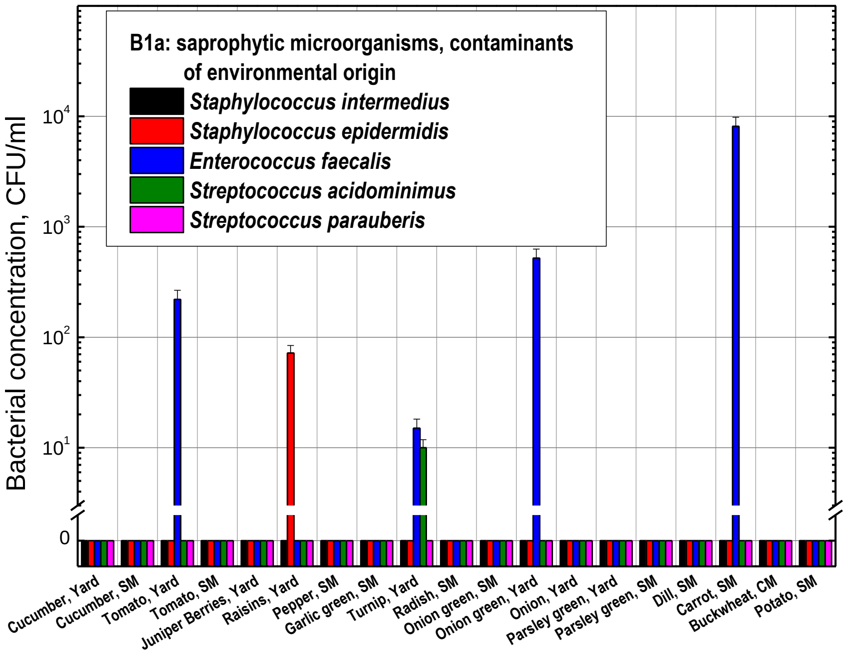


Fig. 22.Key microorganisms isolated from the major plant components of Russian Federation foods: B1a group – environmental saprophytic bacteria.

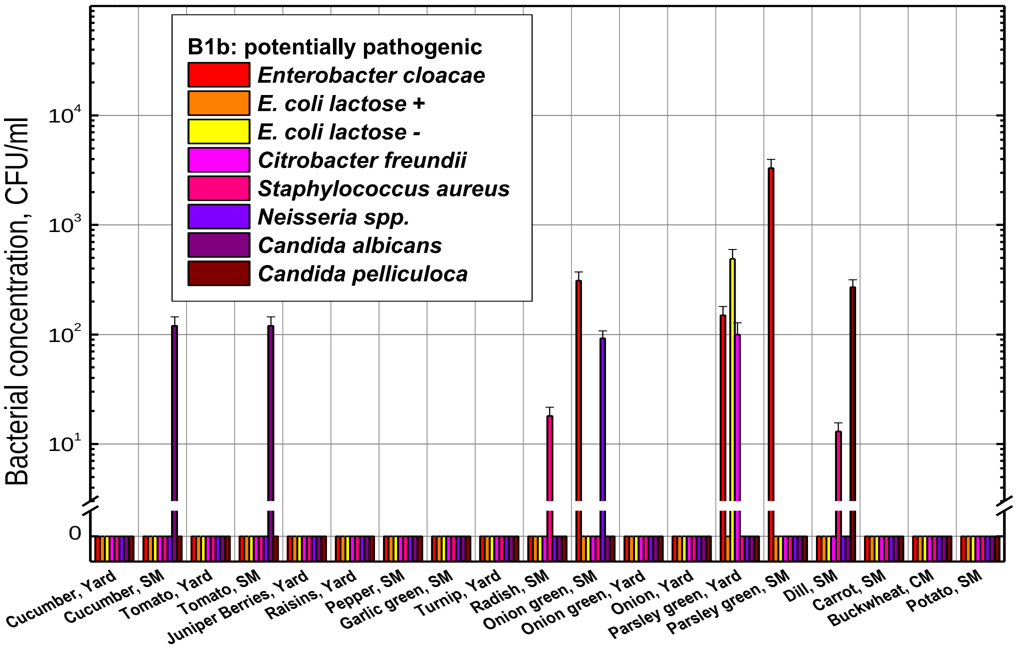


Fig. 23.Key microorganisms isolated from the major plant components of Russian Federation foods: B1b and B2 group – contaminants of human origin.

TURKEY

All the samples were fromcity street markets and dishes were prepared in the Department of Food Technology of YEDITEPE University during the working visit of UzhNU scientistsfrom 5th, October, 2011 until 5th November, 2011 (**Table 8**).The results of microbial assessmentsof major plant ingredients and prioritised foods are presented in **Figs 24 – 27**.

Table 8.Turkish samplesof collected foodsand plant food ingredients selected for the microbiological analysis and their seasonal availability.

No	Plant component	No	Prioritised Foods	Availability
1	Black tea	1	Black tea	Anytime
2	Green pepper	2	Bulgur pilaf	Anytime
3	Siyez bulgur	3	Bulgur pilaf	Anytime
4	Red pepper	4	Bulgur pilaf	Anytime
5	Fruit of the evergreen Cherry Laurel	5	Fruit of the evergreen Cherry Laurel	Late June-Early July
6	Green beans	6	Sautéed pickled green beans	Anytime
7	Corn grits	7	Kale soup	Anytime
8	Dry white beans	8	Kale soup	Anytime
9	Kale	9	Kale soup	Anytime
10	Tomato (paste)	10	Kale soup	Anytime

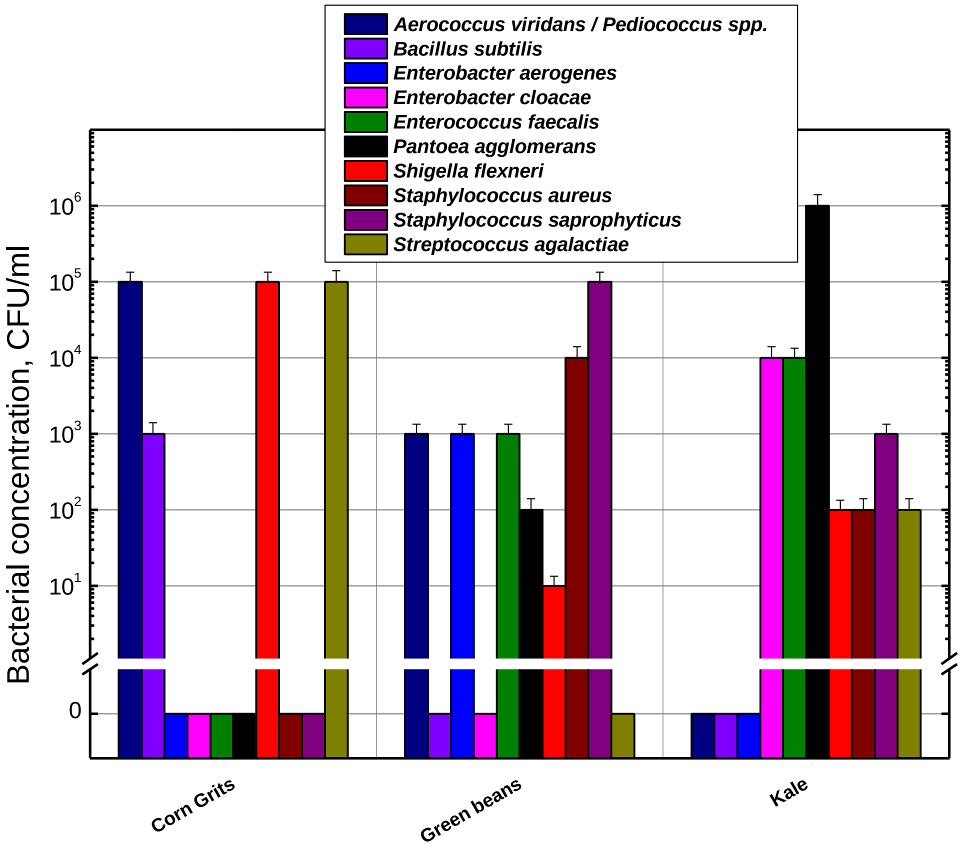


Fig. 24. Variety of bacteria/microorganisms isolated from the Turkish plant and food samples.

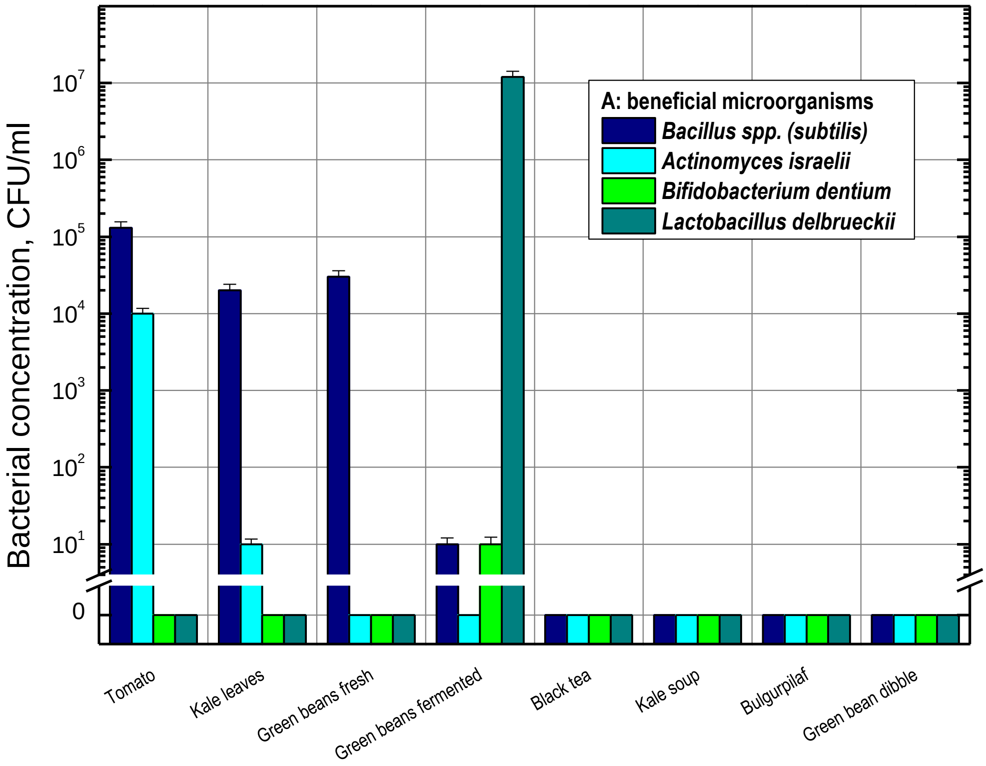


Fig. 25. Beneficial microorganisms in major plant components of Turkishfoods: group A.

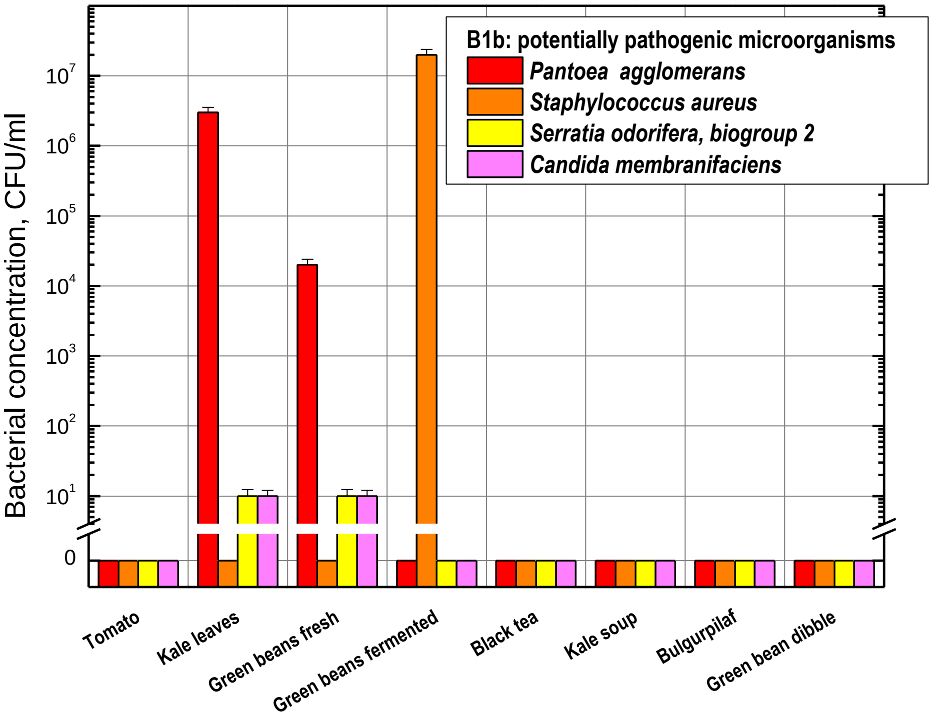


Fig. 26.Key microorganisms isolated from the major plant components of Turkish foods: B1b and B2 group – contaminants of human origin.

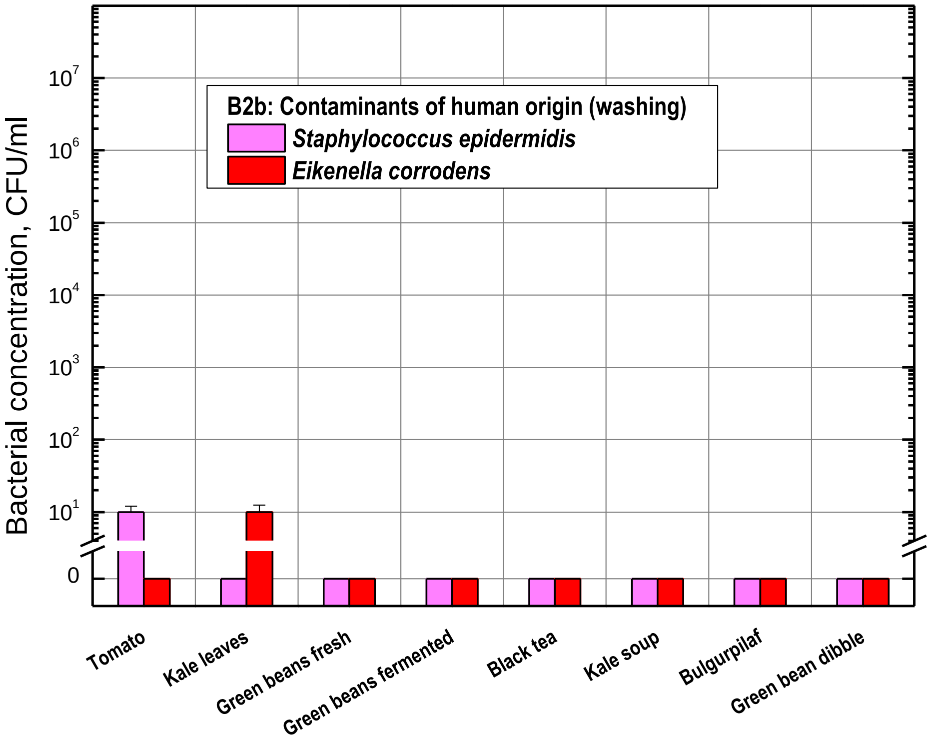


Fig. 27.Representativecontaminants of human origin obtained from Turkish foods during the preparation procedure.

The range of microorganisms isolated from samples provided by YEDITEPE University in 2010 is shown in **Fig. 24**. *Aerococcus viridans*(synonymous is *Pediococcus spp.*) 10^5CFU/ml was found in corn grits and 10^3 CFU/ml in dry green beans; *Bacillus subtilis*was also detected in corn grits - 10^3 CFU/ml, *Enterobacter aerogenes*was found in green beans (10^3CFU/ml) in addition to*Enterococcus faecalis*, in similar amounts,and *Shigella flexneri* (10^1CFU/ml); kale leaves were contaminated by *Pantoea agglomerans* (10^6 CFU/ml), *E. cloacae* (10^4 CFU/ml), *Staphylococcus aureus*(10^2 CFU/ml), *S. saprophyticus*(10^3 CFU/ml), *Streptococcus agalactiae*(10^2 CFU/ml). *S. aureus*were also present in green beans (10^4CFU/ml) together with *S. saprophyticus*(10^5CFU/ml). From sample of corn grits provided by YEDITEPE,*S. agalactiae* 10^2CFU/ml and *S. flexneri* (10^5CFU/ml) have been isolated.

Beneficial bacteria associated with Turkish plant ingredients and foods are shown in **Fig. 25**. *B. subtilis*was found in 10^5CFU/mlin tomatoes, more than 10^4CFU/ml was foundin kale and fresh green beans, and at levels of 10^1in fermented green beans. *Lactobacillus delbrueckii*was the predominant microorganism isolated from fermented green beans (10^7CFU/ml), *Actinomyces israelii*was isolated from tomatoes and kale leaves ($10^4$and 10^1CFU/ml,respectively) and*Bifidobacterium dentium*was detected in very low amounts (10^1CFU/ml) in fermented green beans.

Representatives of the B1a group were not found in the samples and the B1b bacteria groupmainly consisted of*P. agglomerans* (10^4 CFU/ml) in fresh green beans, *S. aureus*(10^7CFU/ml)in fermented green beans, *Serratia odorifera* biogroup 1 (10^1 CFU/ml) and, in the same amount,*Candida membranifaciens* in green beans and in kale leaves.

Finally,*Staphylococcus epidemidis*and *Eikenella corrodens,* whichbelong to the group of B2b microorganisms – contaminants of human origin isolated during the process of foods preparation – were found in 10^1 CFU/ml in both tomatoes and kale leaves.

UKRAINE

The samples of major plant ingredients were collected in three different places: 1) farms (private yards) in the Transcarpathian region; street markets and markets, and food samples including homemade products prepared according to original recipes obtained from cities and villages, and from restaurants using traditional food brands. **Table 9** summarises the results obtained after microbiological estimation of safety and quality of all the Ukrainian prioritised foods and their major plant components. All the plant samples were checked before and after washing during the food preparation.

Group A was mainly represented by *Lactobacilli* and *Bifidobacterium* spp. associated with enterococci and *Bacteroides* (from anaerobes). *Saccharomyces cerevisae* wasnot found in any of the testedplant samples. The category B1a microbial isolates obtained from Ukrainian samples included*Erwinia spp.*, *Xanthomonas spp.*, *Bacillus spp.*and*Pseudomonas spp.The isolated strains of Micrococcus spp., Klebsiella spp*., *Enterobacter spp.*, *Proteus spp.*, *Citrobacter spp*., *Serratia spp.*, *Pseudomonas spp.*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Candida spp.*, *Clostridium spp.* and *Bacteroides spp*. were reported as key microorganisms of B1b group.

Plants and fruits from the city-market had the lowest level of contaminationwith different microorganisms (**Table 9** and **Figs.28 – 30**). Samples from the yard/farmshowed the“typical” environmentalmicroorganisms,mostly represented by *Erwinia herbicola, Xanthomonas campestris,* and *Bacillus subtilis*. The group of microbiota of human origin in these samples was represented by coagulase negative S*taphylococcus species (CNS)*, *E. coli* (lactose-positive and lactose-negative), *Proteus mirabilis* and*Pseudomonas aeruginosa.*

Table 9.Key microorganisms detected in major plant ingredients of prioritized Ukrainian traditional foods (before and after washing)

No	Plant	Source	Place	Isolated microorganisms (native sample)	Isolated microorganisms (washed samples)
1, 2	**Sorrel**	Salovka	Street market	*Coagulase-negative Staphylococcus (CNS)* *Enterobacter aerogenes*	*Proteus mirabilis*
		Esen’		*Shigella flexneri* *Pantoea agglomerans* *Streptococcus parauberi* *Staphylococcus aureus*	*Proteus mirabilis*
3, 4	**Parsley, green**	Rysyno		*Serratia marcescens*	*Proteus mirabilis*
		Dobron’		*Proteus mirabilis*	*Proteus mirabilis*
5, 6	**Parsley, root**	Rysyno		*Pantoea agglomerans* *Bacillus thuringiensis*	*Rodococcus equi*
		Dobron’		*E. aerogenes* *E. cloacae*	
7	**Parsley, green**		City market	*Pseudomonas fluorescens*	*Proteus mirabilis*
8	**Parsley, green**		Yard	*Enterobacter aerogenes* *Bacillus licheniformis*	*CNS* *Bacillus licheniformis*
9, 10	**Potato**	Storozhnytsya	Street market	*Bacillus subtilis*	*Proteus vulgaris* *Shigella flexneri*ABC
		Vynogradovo		*Bacillus subtilis*	*Serratia marcescens*
11	**Potato**		City market	*Proteus vulgaris* *Proteus mirabilis*	*Pseudomonas fluorescens*
12	**Potato**		Yard	*Erwinia herbicola*	
13, 14	**Dill**	Rysyno	Street market	*Serratia marcescens* *Pantoea agglomerans* *Bacillus licheniformis* *Bacillus subtilis*	*Proteus vulgaris*
		Dobron’		*Actinomyces israelii* *Candida pelliculoca*	*Pseudomonas spp.*
15	**Dill**		City market	*Pantoea agglomerans* *Proteus mirabilis*	
16			Yard	*S. saprophyticus* *Bacillus subtilis* *Erwinia herbicola*	*E. coli*
17	**Carrot**	Dobron’	Street market	*Pseudomonas fluorescens* *Pantoea agglomerans* *Bacillus megaterium*	*E. coli*
18		Rysyno		*Bacillus subtilis* *Serratia odorifera* *Xanthomonas campestris, CNS*	*E. coli*
19	**Carrot**		City market	*Erwinia herbicola* *(Pantoea agglomerans)* *Pseudomonas spp.* *Bacillus subtilis*	*Pseudomonas spp.* *CNS*
20	**Carrot**		Yard	*Bacillus subtilis*	
21, 22	**Onion**	Storozhnytsya	Street market	*Pseudomonas fluorescens* *Aerococcus viridans*	*Aerococcus viridans*
		Dobron’		*Bacillus megaterium*	
23		Rysyno		*Eubacterium lentum*	
24	**Onion**		City market	*Bacillus subtilis*	

Table 9. Key microorganisms detected in major plant ingredients of prioritized Ukrainian traditional foods (before and after washing)(cont.).

No	Plant	Origin, place	Type of market	Native, bacterial species (major isolates)	Washed out/bacterial species (major isolates)
25, 26	**Garlic**	Rysyno	Street market	*Erwinia herbicola* *Pantoea agglomerans* *Bacillus megaterium*	*Bacillus megaterium*
		Vynogradovo			*CNS*
27	**Garlic**		City market	*Serratia marcescens biogroup odorifera*	
28, 29	**Beet**	Rakovets’	Street market	*E. coli*	
		Rysyno		*Bacillus subtilis* *Bacillus licheniformis* *Bacillus megaterium*	*Proteus mirabilis* *Bacillus subtilis*
30, 31	**Beet**		City market	*Erwinia herbicola* *Pantoea agglomerans*	*E. coli* *CNS*
			Yard	*Bacillus megaterium* *E. vulneris* *E. coli*	*Micrococcus luteus* *Bacillus megaterium*
32, 33	**Celery**	Dobron’	Street market	*Micrococcus luteus* *Bacillus subtilis*	*Proteus mirabilis* *E. coli*
		Chaslivtsi		*Pantoea agglomerans*	*Bacillus subtilis*
34, 35	**Cabbage**	Storozhnytsya		*Pantoea agglomeran* *Streptococcus acidominimus*	*Proteus mirabilis*
		Lviv		*Enterobacter aerogenes*	*Enterobacter aerogenes* *CNS*
36	**Cabbage**		City market	*Bacillus subtilis*	*Bacillus subtilis* *E. coli*
37	**Cabbage**		Yard	*Bacillus subtilis* *Proteus mirabilis* *Staphylococcus epidermidis*	*CNS*
38	**Tomato**	Rysyno	Street market	*Bacillus subtilis* *Bacillus thuringiensis* *Enterococcus faecium*	*Citrobacter freundii* *Bacillus megaterium*
39	**Tomato**	Vynogradovo		*Aerococcus viridans* *Bacillus thuringiensis* *Bacillus subtilis*	*E. coli* *CNS*
40, 41	**Tomato**		Yard	*Bacillus subtilis*	
	Tomato			*Erwinia herbicola* *Proteus mirabilis*	*CNS*
42	**Beans**	Chaslivtsi	Street market	*Pantoea agglomerans* *CNS* *Bacillus licheniformis* *Erwinia herbicola*	*E. coli* *Bacillus subtilis*
43	**Beans**	Znyatsevo		*Pantoea agglomerans* *Xanthomonas campestris* *Pseudomonas fluorescens*	*CNS* *E. coli*
44	**Beans**		City market	*Bacillus cereus*	*E. coli*
45	**Beans**		Yard	*Erwinia herbicola*	
46, 47	**Cucumber**	Rysyno	Street market	*Proteus mirabilis*	
		Velyki Luchky		*Proteus mirabilis* *Micrococcus luteus* *Enterococcus faecalis*	*E. coli* *S. aureus*
48	**Cucumber**		City market	*Bacillus subtilis* *Proteus mirabilis*	
49	**Cucumber**		Yard	*Erwinia herbicola* *Bacillus subtilis* *Pseudomonas fluorescens*	*Bacillus subtilis* *Pseudomonas fluorescens*

Table 9. Key microorganisms detected in major plant ingredients of prioritized Ukrainian traditional foods (before and after washing) (cont.).

No	Plant	Origin, place	Type of market	Native, bacterial species (major isolates)	Washed out/bacterial species (major isolates)
50	**Apple, fresh**	Patskanyovo	Street market	*Bacillus megaterium*	*CNS* *E.coli*
51	**Apple, fresh**	Znyatsevo		*Pseudomonas fluorescens* *Erwinia herbicola*	
52	**Apple, fresh**		City market	*Pseudomonas fluorescens* *Bacillus subtilis*	*Proteus mirabilis*
53	***Apple (fresh)***		Yard	*Bacillus subtilis* *Staphylococcus intermedius*	*Pseudomonas spp.*
54	**Apple, dry**		City market	*Staphylococcus intermedius*	*Bacillus subtilis*
55	**Nettle**		Yard	*Proteus vulgaris* *Pantoea agglomerans*	*Rodococcus equi* *E. coli*
56	**Plum, fresh**			*Erwinia herbicola*	*CNS*
57	**Grape, fresh**			*Bacillus subtilis*	
58	**Pear, fresh**			*Bacillus licheniformis*	*Enterobacter cloacae*
59	**Pear, fresh**		City market	*Bacillus subtilis*	*CNS*
60	**Pear, dry**			*Bacillus subtilis*	*CNS*
61	**Plum, dry**		City market	*Bacillus subtilis*	
62	**Melon**		City market	*E. coli* *Corynebacterium spp.*	
63	**Melon**		Street market	*Proteus vulgaris* *Pantoea agglomerans* *Enterobacter cloacae*	
64	**Elderberry flowers**		Yard	*Bacillus subtilis* *Enterobacter cloacae*	*S. aureus*
65	**Nettle leaves**		Yard	*Candida albicans* *Enterobacter cloacae*	
66	**Red pepper**		City market	*Bacillus subtilis*	
67	**Yellow-red pepper**		Street market	*Enterococcus cloacae*	
68	**Green (spring) garlic**		City market	*Lactobacillus catenaforme*	*S. aureus*
69	**Green (spring) onion**		Street market	*Enterobacter cloacae*	
70			Yard	*Enterobacter faecalis* *Escherichia hermanii*	

The greatest rangeof microorganisms was found in all the samples obtained from the street-market, due to the fact that these foods are collected and transported from different regions in Transcarpathia. On the other hand, their high contamination with human originated microbiota hasbeen shown. The potentially pathogenic bacteria: *Enterobacter cloacae*, *Proteus vulgaris/mirabilis*, *Streptococcus pneumoniae*, *Str. pyogenes*, *Escherichia* *coli*lactose-negative, etc. including the *Clostridium spp.* and *Bacteroides spp.* together with *Candida albicans* and other microscopic fungi were typically present on all of these plants' surfaces.

Plants from the street market were contaminated by different *Escherichia* spp. (*E. coli* or *E. hermanii*, or *E. vulneris*), *Klebsiella pneumoniae*/*oxytoca* and gram-positive opportunistic microorganisms (*Streptococcus pneumoniae* and *Staphylococcus aureus*). *Shigella flexneri*ABC were obtained from sorrel before, and in potato after, the washing procedure (before cooking). This is an evidence for the human/water source of contamination in the tested sample.Before washing, the predominant isolates were *Klebsiella pneumoniae; Serratia odorifera biogroup 1; Pantoea agglomerans; Enterococcus faecalis* and *Staphylococcus aureus*.

After washing and cutting, the most contaminated samples were potatoes and carrots, and the less contaminated was garlic. This must be due to the presence of its ownendogenous volatilesand antibiotic substances (including allicin). Only garlic was found to contain *Serratia marcescens*. In the samples, after washing and cutting, the levels of bacterial contamination of plants was significantly increased compared to that in the raw (native) material (best examples beingcucumbers, apples and pears). After the first stage of food processing, washing and cutting of plants/fruits/vegetables, the predominantbacterial species were *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus aureus*.

E. coli (EPEC), *Shigella dysenteriae*, *E. coli* O157:H7 and gram-positive food-borne pathogens (*Listeria monocytogenes, Campylobacter jejuni*) were not detected in any of the tested samples.

Concerning the anaerobes isolated from different plants and different sources it can be concluded that: i) they were rarelydetected in leaves (green parts of plants); ii) *Bacteroides ovatus* had been isolated from garlic and tomato from a city market and from tomato purchased in street market (delivered from Vynogradovo) [this sample also contained *Candida albicans*]; iii) *Clostridium butyricum* had been found in parsley (root) and carrot taken from street market and deliveredfrom Dobron' and Rysyno;iv) *Clostridium carnis* contaminated beet from Rysyno, obtained from a street market, as well as beans from a city market; v) *Bifidobacterium longum* were isolated from parsley (green), Rysyno, dill and celery from Dobron'; vi) *Eubacterium lentum* was only detected from onion (street market, Rysyno).

Beneficial bacteria were poorly represented in all the tested samples. In addition to *Bifidobacterium longum* (isolated from parsley (green), Rysyno, dill and celery Dobron'), a few different Lactobacilli strains were detected in low amounts in the following plant samples: garlic, potato and cabbage (Storozhnytsya); carrot and cucumber (Rysyno); celery (B, after washing, Dobron'); cabbage (Lviv), bean (Chaslivtsi); onion (yard); nettle (Uzhhorod, city park); beet (Racotsi); cucumber (Velyki Luchky). Not one plant sample from the city market was contaminated by Bifidobacterium or Lactobacillus species.

Two more genera were also investigated as key microorganisms: *Bacillus subtilis* and *Corynebacteium spp.*They are widely distributed in nature and are mostly innocuous. These bacteria were mainly isolated from roots (not green part, except celery – for Bacillus) and parsley, yard (for Corynebacterium). Corynebacterium was detected as one of the bacterial compositions in plant native surfaces: tomato, bean, cucumber, pepper, pear and plum (yard); apple (fresh) – street market (from Patskanyovo) and melon – from city market.

The comparative results of microorganisms isolated before and after washing in all the samples collected from city markets, street markets and directly from farms are shown in **Figs. 28 – 30.**

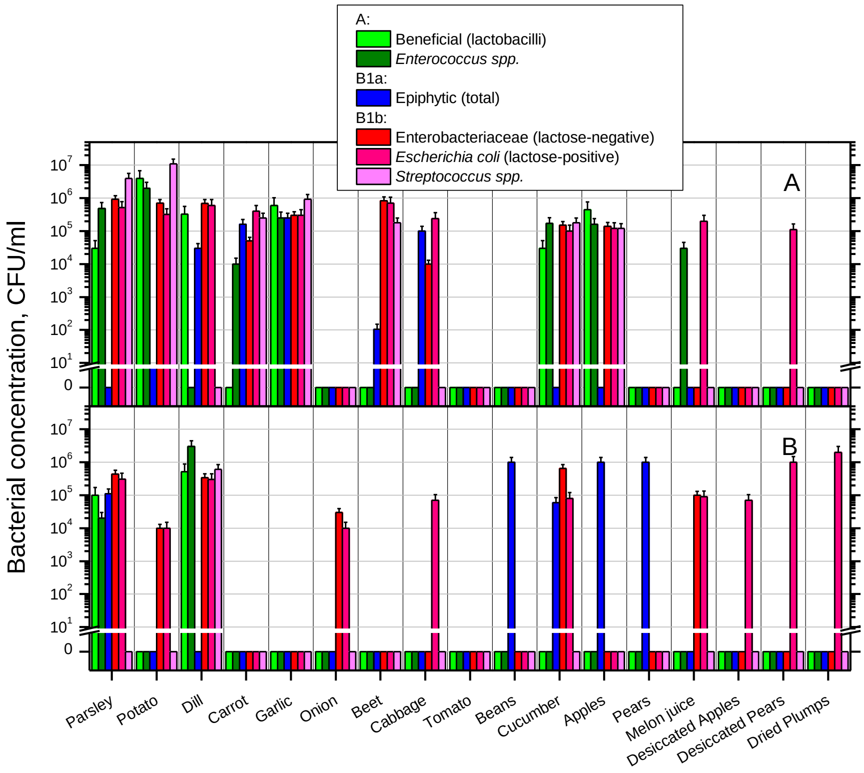


Fig. 28. Dominant groups of microorganisms isolated from Ukrainian plant samples obtained from city markets: A – native samples, before washing; B –washed and sliced samples.

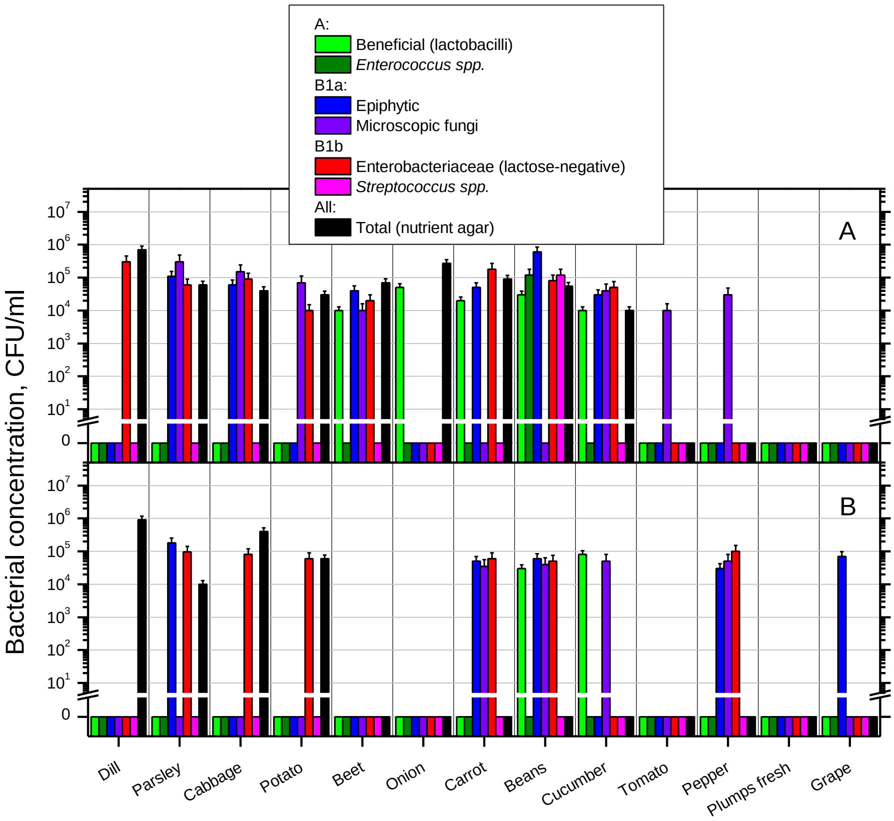


Fig. 29. Dominant groups of microorganisms isolated from Ukrainian plant samples obtained from street markets: A – native samples, before washing; B –washed and sliced samples.

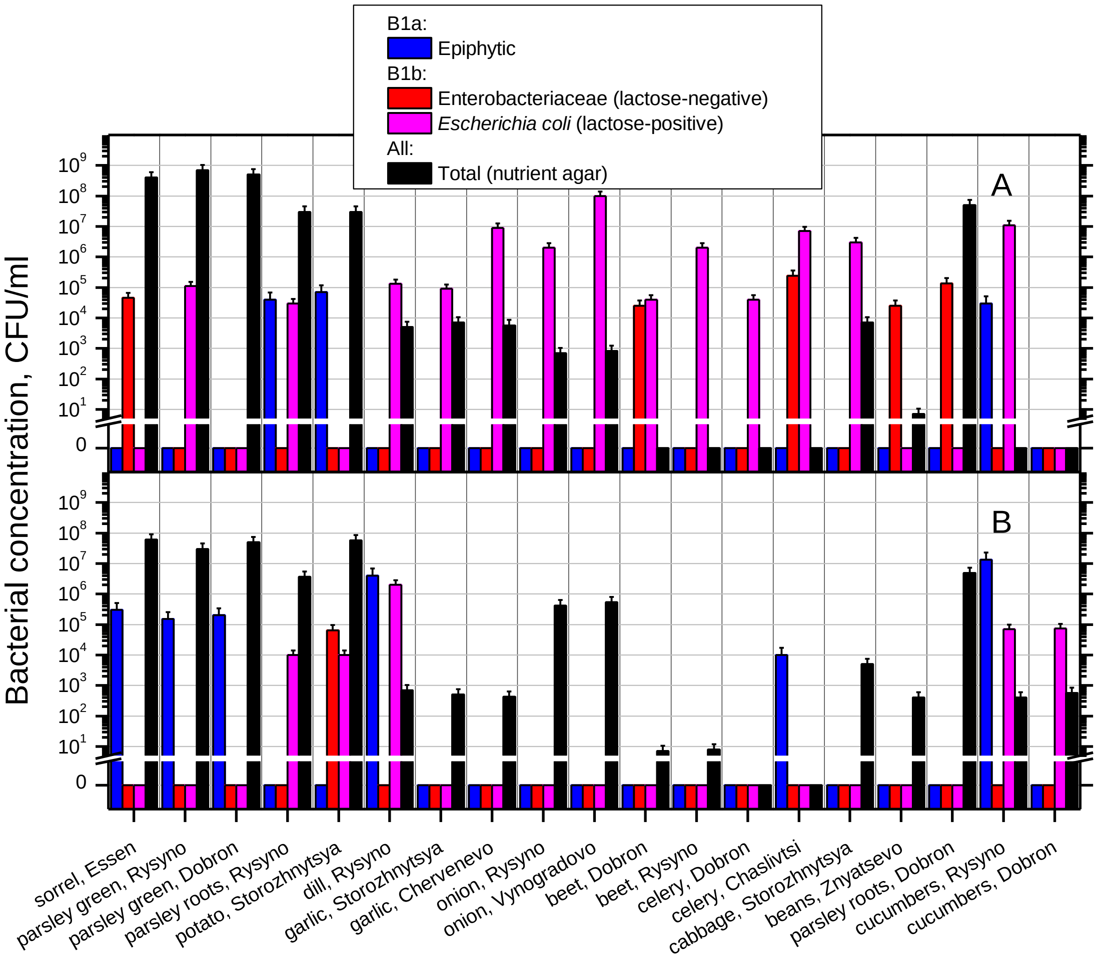


Fig. 30.Dominant groups of microorganisms isolated from Ukrainian plant samples obtained fromfarms [yards]: A – native samples, before washing; B –washed and sliced samples.

Contamination of fruits and vegetables was found to be significantly lower in samples that were washed before plating (in all probes it is sample B) than in those plated in exterior (native) form. This indicates the positive effect of thorough washing of plant-derived products before preparing food and directs their usage in raw form. Vegetables and fruits from different sources are contaminated in a different manner to what was predicted: products/plant-derived samples/foods from supermarkets are less contaminated with microscopic fungi when compared to the same products bought in the street market or grown at home in the yard/field/farm. This may be due to the fact that,before admission to the supermarket (and maybe in the supermarket), products are treated with chemical substances (preservatives) to prolong the shelf life of most fresh products byinhibitingspoilage. At most supermarkets bactericidal lamps are widely used. Correspondingly, it wasfound that in many foods "products" – which tended to beraw materials – purchased in supermarkets contained the following species of microscopic fungi: *Sporothrix cyanescens, Aspergillus nidulans Sporothrix shenckii, Aspergillus flavus, Paecilomyces lilacinus, Scopulariopsis candida, Zygosporium masonii*. Only the one of the samples (tomatoes) was contaminated by *Candida albicans*. The greatest biodiversity of microscopic fungi was observed in samples purchased in the streetmarket. This is probably due to the fact that the products available in such markets are at risk of contamination at a variety of stages in the production chain(including harvesting, processing, shipping, hand packaging, handling by shoppers, etc.). Such market product was contaminated with fungi of the following species: *Sporothrix cyanescens, Aspergillus nidulans, Gymnoascus dankaliensis, Paecilomyces lilacinus,* *Aspergillus niger, Sporothrix shenckii, Scopulariopsis candida, Aspergillus terreus, Fusarium chlamidosporum*. In addition, most of the samples taken from this retail source were contaminated by *Candida spp.* Samples obtained from the private yards/fields/farms were mostly contaminated by microscopic fungi of *Aspergillus nidulans, Fusarium moniliforme, Sporothrix cyanescens*, and only in small proportion of the tested samples were organisms of the genus *Candida*detected (cabbage from Lviv and Storozhnytsya among of them).From the results of estimations of ready-to-eat meal, it can be concluded that most are not contaminated or contaminated with only very small amounts of saprophytic (*Bacillus subtilis, Staphylococcus epidermidis, Pantoea agglomerans*) or opportunistic pathogens (*Enterobacter cloacae* and *Staphylococcus aureus*). All isolated species are presented in **Table 10**. The maximum titer for the bacteria is 10^2 CFU/ml and therefore itisin accordance tocurrent (actual) sanitary standards of food safety. Data for sauerkraut are provided in **D2.5b**.

Table 10. Microorganisms isolated from prioritised Ukrainian traditional foods.

No	Food	Source, place	Microorganism	Titre, CFU/ml
1, 2, 3	**Green Borsch (with sorrel)**	Homemade: incity and villages	*Bacillus subtilis*	$2,0 \cdot 10^{1}$
				$3,0 \cdot 10^{1}$
				$2,5 \cdot 10^{1}$
			Staphylococcus epidermidis	$1,0 \cdot 10^{1}$
4, 5, 6, 7		Restaurant	*Bacillus subtilis*	$3,0 \cdot 10^{1}$
				$4,0 \cdot 10^{1}$
				$1,0 \cdot 10^{1}$
				$8,0 \cdot 10^{1}$
8, 9, 10	**Red Borsch (with beet)**	Homemade: incity and villages	*Bacillus subtilis*	$3,5 \cdot 10^{2}$
				$0,9 \cdot 10^{3}$
				$2,8 \cdot 10^{1}$
11, 12, 13		Restaurant		$3,0 \cdot 10^{1}$
				0
				0
14, 15, 16	**Uzvar**	Homemade: in city and villages	*Bacillus subtilis*	$1,0 \cdot 10^{1}$
				$1,0 \cdot 10^{1}$
				0
17, 18, 19		Restaurant		0
				$1,0 \cdot 10^{1}$
				$1,0 \cdot 10^{1}$
20, 21, 22	**Pomazanka (with dill)**	Homemade: in city and villages	*Enterobacter cloacae*	$2,0 \cdot 10^{0}$
				$9,0 \cdot 10^{2}$
				0
			Staphylococcus epidermidis	$4,0 \cdot 10^{0}$
				$6,0 \cdot 10^{0}$
				$1,0 \cdot 10^{1}$
23		Restaurant	*Staphylococcus aureus*	$1,0 \cdot 10^{0}$
			Staphylococcus epidermidis	$4,0 \cdot 10^{0}$
24, 25			*Enterobacter faecalis*	$2,0 \cdot 10^{0}$
				$1,0 \cdot 10^{1}$
26, 27, 28	**Roasted sunflower seeds**	Homemade	*Bacillus subtilis*	$1,0 \cdot 10^{2}$
				0
				0
29, 30		City market, two different local brands	Any cultivated	0

DISCUSSION

Comparable data from microbial assays are presented in **Figs 31 – 36,** where all the targeted groups of key microorganisms relevant to the safety and quality issues that have been prioritised withinthe BaSeFood project,for traditional foods and their major plant components are presented as a percentage ratio for each of Black Sea region country.

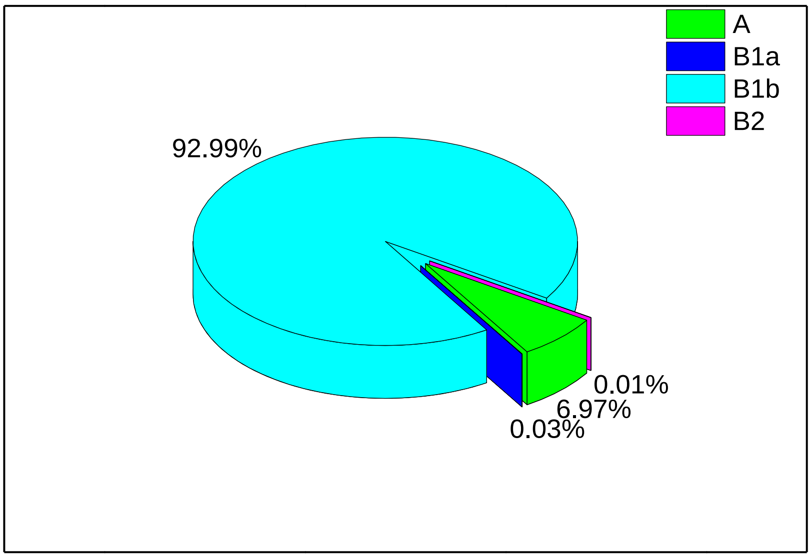


Fig. 31.Ratio between microorganisms: beneficial, contaminants of environmental and human origin and food-borne pathogens: Bulgaria.

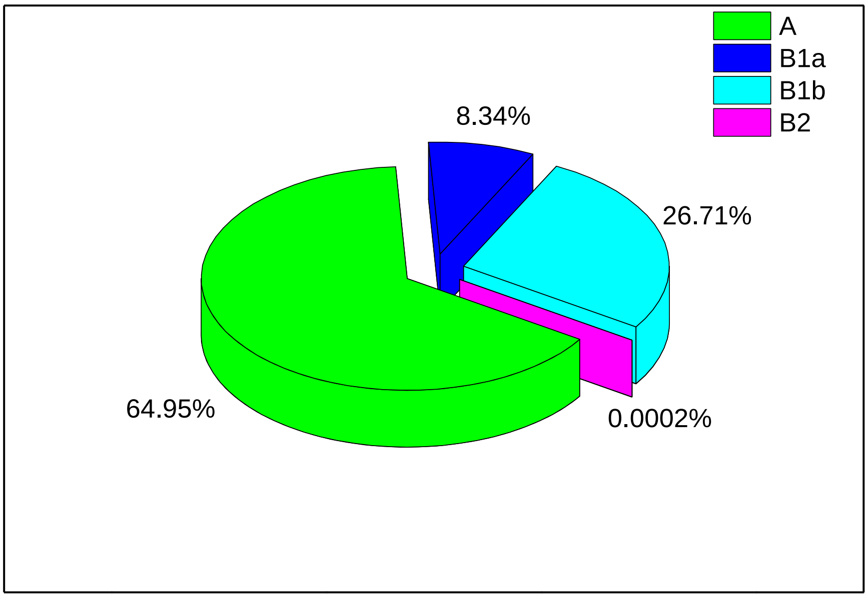


Fig. 32.Ratio between microorganisms: beneficial, contaminants of environmental and human origin and food-borne pathogens: Georgia.

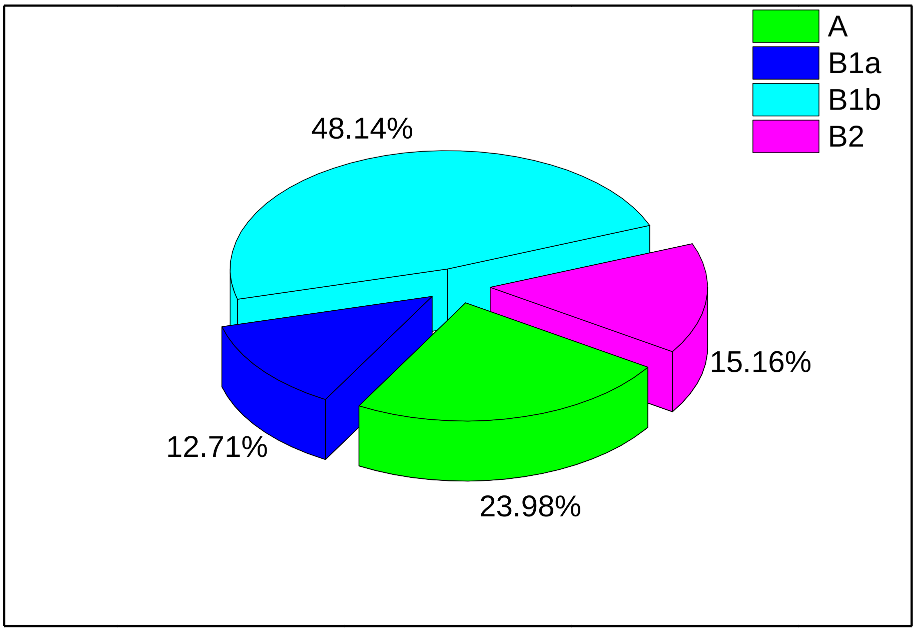


Fig. 33.Ratio between microorganisms: beneficial, contaminants of environmental and human origin and food-borne pathogens: Romania.

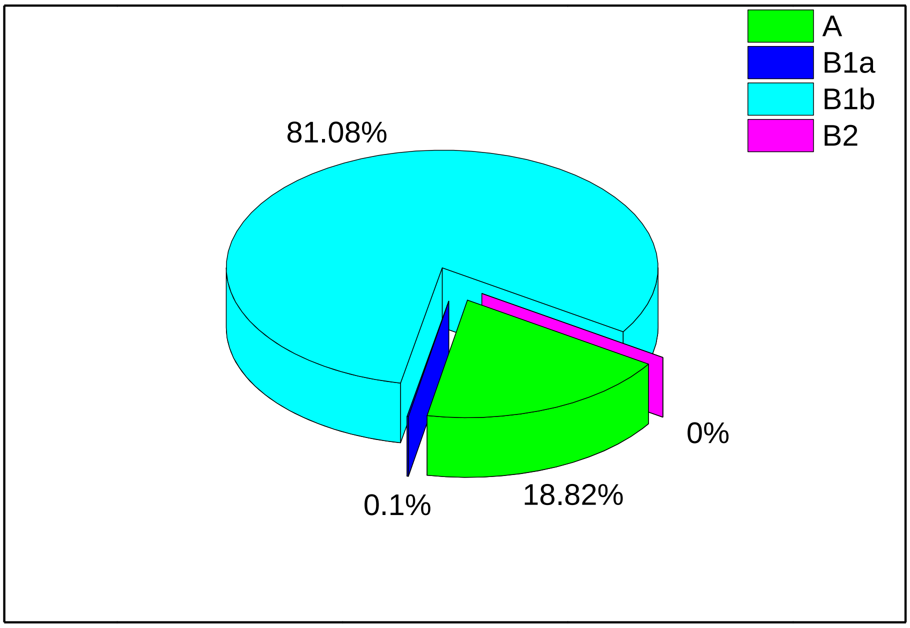


Fig. 34.Ratio between microorganisms: beneficial, contaminants of environmental and human origin and food-borne pathogens: Russian Federation.

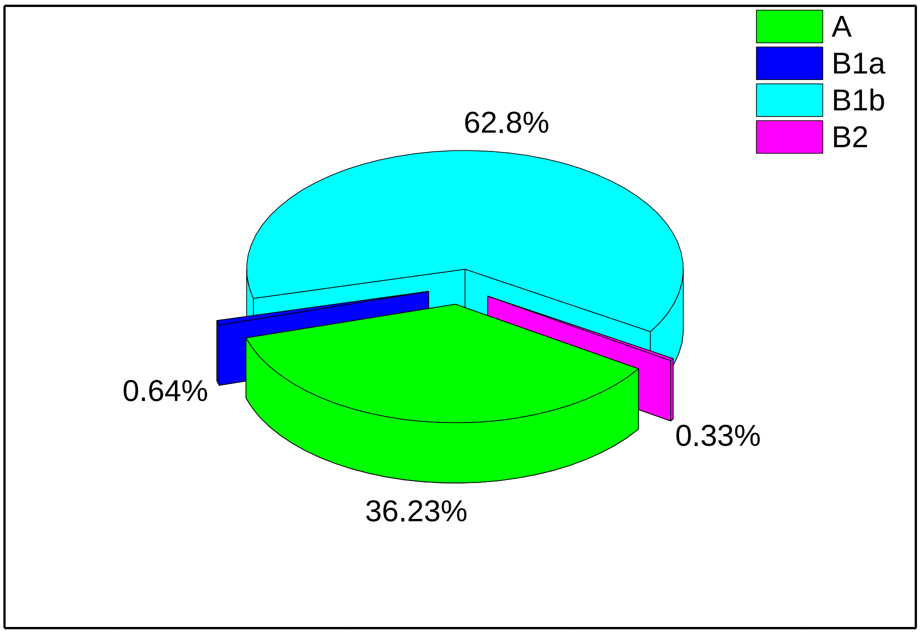


Fig. 35.Ratio between microorganisms: beneficial, contaminants of environmental and human origin and food-borne pathogens: Turkey.

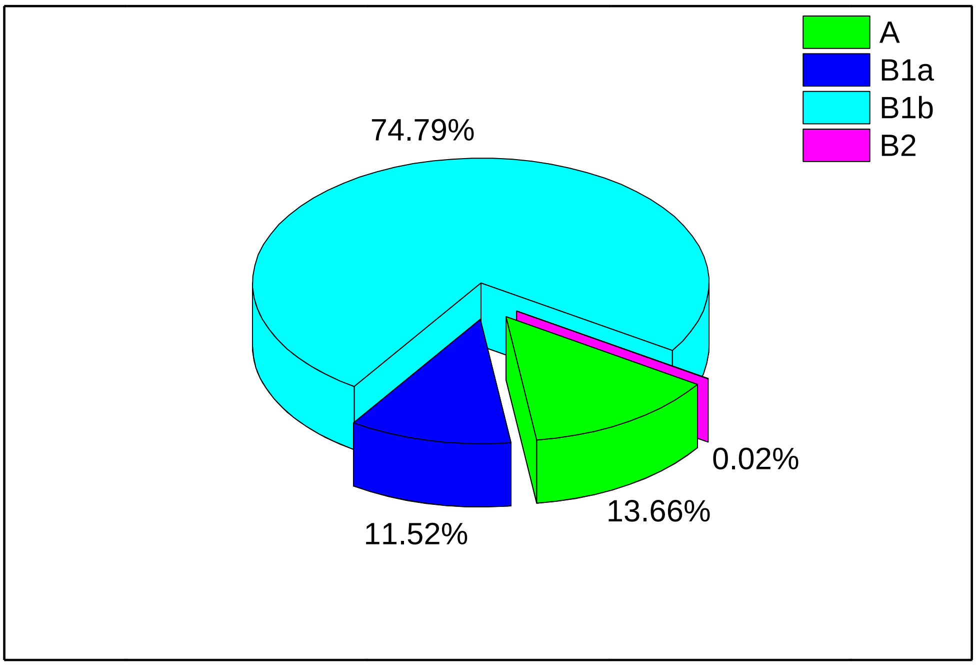


Fig. 36.Ratio between microorganisms: beneficial, contaminants of environmental and human origin and food-borne pathogens:Ukraine.

As can be seen in Fig. 31, the majority of isolates from plants and plantcomponents in Bulgaria belong to group B1b - potentially pathogenic bacteria and contaminants of human origin (up to 90), whilst the richestsource of group A, beneficial bacteria, (up to 7) in this country are fermented products – mainly bosa. The remaining two groups of targeted key microorganisms only account for 1-2% of the total amount of isolates. These patterns are thought to be typical for the other countries of BSAC, except Georgia (Fig. 32).The most similar ratio for the distribution of key-microorganisms was observed (Fig. 34) for Russian Federation plant samples and foods: 81% (B1b) vs. 19% (A), in Ukraine (Fig. 36): 75% (B1b) vs. 14% (A) and Turkey (Fig. 35): 63% vs. 36%. Other types of distribution of key-microorganisms are found in Romania (Fig. 33) – where all the targeted groups of keymicroorganisms were more equally represented: 48% (B1b); 23% (A), 13% (B1a, contaminants of environmental origin) and 16% (B2, potentially dangerous, food-borne pathogenic species). Finally, in Georgia the most representative group was that of beneficial bacteria – up to 65% of isolated strains; 27% belonged to group B1b, approximately 8 % - to B1a, and less than 1 % had been defined as key-microorganisms of the B2 group.

Concerning to the variety of the isolated species, it was found that group A is was mainly presented by Lactobacillus genera: *L. plantarum*, *L. fermentum*, *L. delbrueckii*, *L. catenaforme*, *L. casei*, *L. lactis* and other less common for plant ingredients species of lactobacilli (*L. acidophilus*); Bifidobacterium: *B. bifidum*, *B. dentium* and *B. longum*; Bacillus: *B. subtilis*, *B. licheniformis*; Enterococcus: *E. faecium*. Other genera were rather rarely presented: *Actinomyces israelii*, *Sacharomyces cerevisae*, and *Corynebacterium glutamicum*.These data are similar to results presented in recent publications. For example it was observed that the same species of lactobacilli are also typical for other fermented and dairy products and are often used as probiotic or starter cultures [26-30]. The usage of *L. plantarum* as a starter culture for Thai-style fermented pork sausage, made of minced pork, boiled pork rinds, cooked rice, garlic, salt, sugar, pepper, chili and sodium nitrite, has also been reported.This product is packed in banana leaves or plastic sheets, and is allowed to ferment for approximately 3-4 days at roomtemperature (about 30-35 ^{0}C, in Thailand) [31]. The bacterial and yeast cultures are able to control coliforms and molds[32],as well as promotingan increased content of folate in fermenting melon juice [33].Finally, the *Candida famata*R 28 strain isolated from soy sauce mash is characterized as an effective agent in microbial processes of monosaccharide production [34]. *Candida famata* is a species of fungal yeast related to *Candida albicans*. This yeast is not usually responsible for common yeast infections, although it has been isolated from a patient with acute zonal occult outer retinopathy [35].

Concerning the B1b group of bacteria – those of environmental origin, theseare mainly represented by epiphytic bacterial and fungal species. In this research very limited variety and low amounts of such microbial genera: mainly,*Xantomonas (campestris)*, *Pseudomonas (fluorescens)* and *Corynebacterium (tumefaciens)*have been found.On rare occasions,*Erwinia herbicola*and other typical epiphytic species were identified. Similar results are reported in “pathogencombat” project [36],in which the main microbial contamination of all food raw materials,including vegetables and fruitswas studied. Medicinal plants are also very often the subject of microbial assessment. Recently it was reported [37] that chamomile, leaves of orange tree, flowers of linden, corn silk, marine alga, pennyroyal mint and garden sage are usually contaminated with *Bacillus cereus*,whilst spores of *Clostridium perfringens* were detected in the great majority (83.9%) of samples. The mean level of fungal population was $10^{5.5}$ CFU/g and many types of yeast (*Fusarium* spp., *Penicillium* spp., *Aspergillus flavus* and *Asp. niger*) were found, but the predominant species were *Cryptococcus laurentii* (28.1%) and *Rhodotorula mucilaginosa* (22.8%). The mean level of *Crypt.laurentii* contamination in corn silk was greater than 10^4 CFU/g. In this study,*Clostridium perfringens* and *Bacillus cereus* were only rarely detected. The “resistance” of some plants, for example, nettle, to microbial contaminants can be explained by their antimicrobial properties [38], eventhough they still might be a source of classical epiphytic bacteria – *Ralstonia (Pseudomonas) solanacearum*

Race 3 (Biovar 2) [39]. There are many reports that epiphytic bacteria have protective role against phyto-pathogenic bacteria. One of these, *Pseudomonas fluorescens,* has been usedas a potential biocontrol agent [40]. Endophytic bacteria in root and stem tissues of black pepper *(Piper nigrum L.)* are active against *Phytophthora capsici* [41]. *Pediococcus* genera representatives can persist on plant surface as bacteriocines [42].

Finally five vegetables, *Lycopersicon esculentum* (tomato), *Allium cepa* (onions), *Capsicum annum* (pepper), *Telfairia occidentalis* (fluted pumpkin leaves) and *Cucumis sativus* (Cucumber) were purchased from ***various markets*** in Calabar metropolis (Nigeria) and studied to determine their level of bacterial contamination and safety for human consumption. It was shown that the main isolated strains belonged to the following Genera and species: *Bacillus* 83(54.52%), *Micrococcus* 2(1.31%), *Escherichia coli 36(23.53%), Proteus vulgaris 4(9.80%), Lactobacillus acidophilus 7(4.56%), Enterobacter aerogenes 15(9.80%)* and *Alcaligen faecalis 6(3.92%)*. *Bacillus* species and *E. coli* were isolated from all the vegetables. It was concluded in this paper [43], that raw vegetables from these markets are unfit for human consumption and adequate cooking with proper handling before consumption is strongly recommended. In this study, the dominant contaminants were potentially pathogenic bacteria, while less of the beneficial group organisms were found; the majority of the tested samples of raw fruits and vegetables in city markets were, in general, less contaminated compared to those from street markets.

Mycotoxins in food are another important source of human food-borne (intestinal) illness. A recently published review has addressed the role of these secondary metabolites produced by microscopic fungi that are able to cause human disease and, at the same time, are widely distributed in the environment – including plant leaves and surfaces [44].

The most important mycotoxins associated with human diseases are aflatoxin, citrinin, ergot akaloids, fumonisins, ochratoxin A, patulin, trichothecenes, and zearalenone (see also D2.2). Correspondingly, the major species of microscopic fungi known to produce these metabolites are representatives of *Fusarium spp*., *Penicillium spp.*, *Aspegillus spp.* etc[45].

Usually, it was found that plant samples which are contaminated with such microorganisms are free of mycotoxins. There is no really well-studied mechanism or described/defined conditions that stimulate or lead to the production and accumulation of toxic metabolites for human and animal hosts. In a similar manner to virulent bacteria, the ability to produce mycotoxins is mainly defined by specific features of each strain.This means that the isolation/detection of species of microscopic fungi that have potential to produce mycotoxins *cannot be accepted* as evidence of their metabolites in plant/food samples. An important (physiological) role of fungal metabolites is inhibition of bacterial growth – as in the case of antibiotics or in protecting plants from the phyto-pathogenic species of fungi [46]. The results presented here demonstrate that there are different species of microscopic fungi that can be isolated from plant samples. It is noteworthy that *Penicillium spp.*are not listed amongst the major contaminants. *Candida* species are also very rarely found,even though Candida genera is very heterogeneous with some species often being used in biotechnological processes [47], as well as widely presented in fermented products (see D2.5b). Our results support the very wise opinion of the necessity of using only reliable biomarkers for food quality and safety assays [48-50]. In the research presented here,QuickTox™ tests have only been applied in cases when *Fusarium*, *Alternaria*, *Aspergillus* or *Penicillium* spp. have been isolated. No toxins (deoxynivalenol, alternariol, aflatoxin, and ochratoxin A) were identified. These results are expected since the ability of microscopic fungi to produce these toxins has mainly been reported in products prepared/cooked from fruits and grains which have been attacked by moulds in the field prior to their harvesting or under storage conditions.

The special attention paid to potentially pathogenic bacteria that can cause the so-called “opportunistic” infections is justified by the fact that thesebacteria are dominative etiological agents of wide spectrum of human pathology [51-73], mainly due to their resistance to different external factors and antibiotics. In this study, all isolated potentially pathogenic species have been divided into two groups according to their source (origin) of samples contamination: environmental – B1b group and via washing in food processing/preparation. These bacteria, which were also found as contaminants of tested plant foods ingredientsare presented by some gram positive species: *Staphylococcus* (*aureus*), *Streptococcus* (*pyogenes*) and gram negative: *Klebsiella* (*pneumoniae, oxytoca*), *Proteus* (*vulgaris, mirabilis*), *Pseudomonas* (*aeruginosa*), *Enterobacter*(*cloacae*) [51]. The listed bacteria are important and crucial in clinical practice; thus, in recent years *Staphylococcus auricularis sp. nov.* [52], *Aerococcus viridans*[53 – 57],*Pantoea agglomerans*[58 – 65], *Serratia marcescens* [66 – 69] and *Serratia odorifera biogroup1* [70]have all been intensively reported as responsible for the human outbreaks including endocarditis (*Aerococcus viridans* associated). Micrococcus spp. representatives, isolated from tested plant components possibly only accidentally can be relevant to nosocomial infection [72]. Only in immune-compromised oncology patients can typical representatives of epiphytic microbiota, like *Pseudomonas fluorescens* cause bacteremia [73].

The different *in vitro* and *in vivo*pathogenic properties of isolates belonging to the group of potentially pathogenic bacteria were tested to determine their susceptibility to modern antibiotics (data not presented in this report). Not one of the tested strains had the ability to cause the “productive” infection in the mouse model used in doses of 100, 250 and 500 ($\cdot 10^6$) CFU/ml; at the same time, these microorganisms were resistant to the spectrum of antibiotics employed (amino penicillin). *E. corrodens*, *P. rettgeri*, *E. coli*, *P. agglomerans*produced extended spectrum beta-lactamases (ESBL). Strains of *Pantoea agglomerans*, isolated from plants in three different countries (Ukraine, Turkey and Bulgaria), characterized by the same resistance to the largest number of tested antibiotics, particularly to ampicillin, amoksatsylinklavunat, cefotaxime and ceftazidime. *Serratia odorifera biogroup* 1 and *Klebsiella oxytoca* were susceptible to most of the studied antibiotics, except for ampicillin. Antibiotics aminoglycoside and ciprofloxacin were able to inhibit growth of all tested bacteria.Taking into account that there are numerous reports demonstrating the etiological role of such “non-pathogenic” bacteria in human diseases, this observation is extremely important. The additional risk of contamination of plant/food with such pathogenic bacteria is connected to low quality of food [74].

Both the binding and persistence of microbial species are plant specific. The data presented here is in good agreement with published data [75],regardingthe mechanisms involved in concurrent aggregates deriving from resident and immigrants bacteria.

Concerning anaerobes, it should be emphasized that plant contaminations with “obligatory” pathogenic anaerobes were not observed; at the same time some of these organisms: *Bacteroides ovatus* (predominant commensal intestinal microbe which can cause systemic antibody response in IBD), and *Eubacterium lentum* (*Eggerthella lenta*), found in the human colon and feces, and have been implicated as a cause of ulcerative colitis, liver and anal abscesses, and systemic bacteremia species can be better treated as potentially pathogenic than as saprophytic species.

In this study,the most frequent contaminants isolated during food preparation belonged to *E. coli, P. vulgaris* and *S. aureus*. These are transferred from human skin microbiota and are present in water used in processing or washing [76].

The occurrence of the most dangerous group of microorganisms – B2 – food-borne pathogens was rare and these were accidentally isolated in low amounts of microbial cells. The most possible source of these species is raw material [77]. It has also been reported that pet treats and raw pet food can be sources pathogenic agents [78].

These research data are important for the practical implementation of scientific results. All the methods proposed for food preservation and sterilisation [79 – 84] need to be carefully selected and then applied effectively. Highly relevant to this issue are papers demonstrating the dependence of food composition and microbial changes that occur during processing and preparation [85]. Putting this process on well-defined theoretical and scientific bases will enable the significant improvement of food quality, as well as effectively addressing the safety of food [86].

Finally, microbial monitoring of traditional foods, as well as their major plant components from BSAC have resulted in collection of the most valuable and original strains of microorganisms which can be used in industry, particularly as probiotics and starter cultures. The targets of further research are conformed by several recent publications [87 – 89]. Traditional foods can also be used as an important source of prebiotic compounds [90]. The complexity of the task of wide practical implementation of innovative food products requires the necessity of providing convincing evidence of their positive and specific influence on human health [91], and correct identification of isolated microorganisms [92, 93]. It should be also understood that not only “traditional” LAB bacteria can be exploited as the best probiotics. There are other genera and species of very potential and promising microbial species associated with plant originated and fermented foods [94] and their properties need to be further studied and analysed. Finally, the safety and quality of street foods is an interesting subject for further research [95].

CONCLUSION

1. The ratio and range of species within the isolated and identified microorganisms (groups A/B1/B2) are different in each country, but the main common features are: 1. Theamount of beneficial bacteria and microscopic fungi (A) was usually less than that of isolates from other tested groups; 2. The most frequent organisms found were potentially pathogenic bacteria (B1b), followed by typical plant epiphytic bacteria (B1a); foodborne pathogens (B2) are only rarely found (Table 11).
2. Samples collected from street markets – major plant ingredients of the prioritised traditional foods - were more contaminated with microorganisms than were the samples collected from city markets or taken from private yards/farms. The biological diversity of the microbial species was inherently characteristic of samples from street markets. Only a limited number of species was found in samples from city markets but these were isolated in significant titers.
3. The highest level of microorganisms was observed in green parts of plants and in roots (parsley, sorrel, dill) and the lowest was measured in fresh fruits (apples, pears) and vegetables (tomatoes, peppers, beans). These findings are likely to be related to the structural architecture of the plant surface and by the biological specificity of the sample itself.
4. There are plants and fruits with endogenous antimicrobial properties which, as a result, are not contaminated because of the high content or high activity of these biological active substances. These include: wild plum, pomegranate, onion, garlic, rice, basil leaves dray, sage leaves, wheat (flour), sunflower seeds (city market), greenplums, tomatoes (city market), red cabbage, and spices: poppy seeds, coriander, chili, fennel, mint, black sesame, saffron, bay leaf.
5. Beneficial key microorganisms, included in group A,were isolated from plant samples including(by genera): *Bifidobacterium (dentium, longum)*, *Lactobacillus (plantarum, catenaforme)*, *Bacillus (subtilis, licheniformis)*, *Enterococcus (faecium)*, and *Corynebacterium (fascians).Bifidobacterium longum* (isolated from parsley (green), dill and celery). A few different Lactobacilli strains were found,in low amount, in the following plant samples: garlic, potato and cabbage; carrot and cucumber; celery; cabbage; beans; onion; nettle; beat; cucumber. *Bifidobacterium* species was not isolated from plant samples from city markets, but was found in pepper from farms. Two more genera were also investigated as key microorganisms: *Bacillus spp.* and *Corynebacteium spp.* Bacillus – celery; mainly roots; *Corynebacterium*was detected as one of the bacterial compositions in plant native surfaces: tomato, bean, cucumber, pepper, pear and plum; apple (fresh); melon.
6. A small number of B1a saprophytic-epiphytic microorganisms were isolated from all the tested samples due to their displacement with B1b representatives and were mainly*Xanthomonas (campestris)*, *Erwinia herbicola* and *Pseudomonas (fluorescens)*isolates.
7. B1b was the dominant group of other tested key-microorganisms, except for samples from Georgia (where analysis of plant samples is still in progress; the final visit to examine the plants collected from different sources is scheduled for June, 28th- 5th July, 2012). Key-microorganisms in this group most frequently included different species of *Staphylococcus*, *Streptococcus* genera and various strains of *Enterococcus (faecalis)*, *Aerococcus viridans*, *Pantoea* (*agglomerans)*, Serratia, *E. coli*, *Klebsiella (pneumoniae)*, *Proteus (vulgaris)*, *Enterobacter (cloacae)*, Acinetobacter baumannii, *Bacillus cereus*, *Clostridium* (carnis) and *Candida albicans*.
8. Strains of potentially pathogenic bacteria (*Serratia odorifera* biogroup 1, *Eikenella corrodens*, *Escherichia coli*, *Providencia rettgeri*, *Klebsiella oxytoca, Pantoea agglomerans*) isolated from plants had been investigated *in vitro* for their pathogenic (coagulase, destruction of lecithin, hemolytic) properties and sensitivity to antibiotics (ampicillin(10mg), amoksatsylinklavunat(20/10 mg), cefotaxime(10mg), ceftazidime(30mcg), ciprofloxacin (5mcg), amikacin(30micrograms) and gentamicin (10mg). Only a limited

number of the strains were found to contain such virulence factors; but all strains were resistant to the majority of the antibiotics used. *E. corrodens*, *P. rettgeri*, *E. coli*, *P. agglomerans* were positive for ESBL-production. All these strains were unable to induce productive infection after oral administration in dosages of 100, 250 and 500 (x 10^6) CFU/ml in the mouse (BALB/c) model.

9. Representatives of the B2group (classical food-borne pathogens) included*Salmonella enterica*,identified in sorrel (Ukraine), *Shigella flexneri*ABC from kale, crop and green beans (Turkey), and *Listeria monocytogenes* from bread (Georgia).
10. Anaerobes were not detected in leaves (green parts of plants); *Bacteroides ovatus*was isolated from garlic and tomato samples purchased in city and street markets and was supplemented with *Candida spp*. *Clostridium butyricum* was found in parsley (root) and carrot taken from street markets; *Clostridium carnis* contaminated beet, from street markets, as well as beans from city markets; *Eubacterium lentum* was detected as the only isolate from onion (street market).
11. Plants were contaminated with species of *microscopic fungi*: *Sporothrix cyanescens, Aspergillus nidulans Sporothrix shenckii, Aspergillus flavus, Paecilomyces lilacinus, Scopulariopsis candida, Zygosporium masonii*. Only one of the tomatosamples was contaminated by *Candida spp*. The greatest biodiversity of microscopic fungi was observed in samples purchased from street-markets*: Sporothrix cyanescens, Aspergillus nidulans, Gymnoascus dankaliensis, Paecilomyces lilacinus, Aspergillus niger, Sporothrix shenckii, Scopulariopsis candida, Aspergillus terreus*and *Fusarium chlamidosporum*. Most of the samples taken from such places were contaminated by *Candida spp.* Samples obtained from the private yard/fields were mostly contaminated by microscopic fungi of *Aspergillus nidulans, Fusarium moniliforme, Sporothrix cyanescens*, whilst only in a very low number of samples werefound isolates belonging to the different species of the genus *Candida spp*.
12. The ready-to-eat meals prioritized were either not contaminated with any of the microorganisms (plum and rose jam, plum and pomegranate sauce, flax and mustard oil, tahini pasta and halva, churchhella, nettle with walnut, bulgur pilaf, black tea, kale soup, humus) or were contaminated with very low amounts (up to 10^2 CFU/ml)of *Bacillus subtilis, Staphylococcus epidermidis, S. aureus, Enterobacter cloacae, Enterobacter faecalis, Pantoea agglomerans and Trichosporon spp.*: borsch, sunflower roasted seeds, etc). Due to numerous literature reports about the of ability of these opportunistic pathogens to cause nosocomial outbreaks, their contamination of fresh food should be prevented, and not only the safety issues but also those relating to the quality of the food should be investigated.
13. There is a difference between microbial species isolated from industrial (street markets, restaurants) and homemade products. It was shown that only homemade or original recipes of ***unfermented*** foods - corba, herbal dish, original okroshka, pomazanka, and melon juice contained beneficial bacteria.
14. The richest source of beneficial bacteria is the fermented products. The density of bacteria with potential beneficial properties was significantly higher in the analysed traditional fermented foods and drinks: *L. fermentum, B. breve* and *L. acidophylus*(in boza), *B. dentinum* (in fermented beans, Turkey),*A. israeli, L. plantarum* and *casei* (kvass, Russia).Bacteria/microorganisms originated from fermented foods of homemade traditional dishes/beverages are a matter of great importance, since they can be potentially exploited in industrial food processing. The main bacteria of the fermented dishes/drinks prioritised within the BaSeFood project hasbeen investigated in relation to their preparation processes (see D**2.5b**).
15. There is a difference between the main isolates present at the beginning, during the process and at the end of the fermentation. These strains are undergoing further testing,so as to be certified as potentially valuable industrial strains.

Table 11. Distribution of key-groups of microorganisms in traditional food samples of BSAC and its major plant components.

Foods, amount	Plant components	Country	Isolates: A1+A2/B1/B2	Notes
6	14	Bulgaria	9+11/26/0	0
6/8	12	Georgia	7+8/18/1	*Listeria monocytogenes*
6/7	26	Romania	2/22/2	*Staphylococcus aureus*
6/9	19	Russia	7/42/1	*Staphylococcus aureus*
6/18	28	Turkey	4/7/4	*Shigella flexneri*
6/15	70	Ukraine	18/44/48+4	*Shigella flexneri* *Salmonella enterica*

DISSEMINATION

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Annex 1. *List of analyzed foods and their Plant Components.*

BULGARIA

№	**Plants:**
1	Barley
2	Carrot
3	Flour
4	Good king Henry roots
5	Hot pepper
6	Mint
7	Onion
8	Pumpkin
9	Rose flowers
10	Savoury
11	Smilyan beans
12	Tomato
13	Walnuts
14	Wheat

№	**Food:**
1	Boza
2	Tikvenik (pumpkin pie)
3	Rose jam
4	Rhodopian dried beans
5	Sunflowers seeds
6	Tahan halva
Total	**20**

GEORGIA

№	**Plants:**
1	Wheat flour (Tsiteli Doli variety)
2	Makhobeli seeds
3	Nettle
4	Walnut
5	Grape juice
6	Fresh fruits
7	Wild plums red and green
8	Garlic
9	Red pepper
10	Coriander
11	Fennel
12	Pennyroyal

№	**Food:**
1	Black tea
2	Bread
3	Green tea
4	Long Bread
5	Nettle with walnut
6	Tsiteli doli bread with makhobeli
7	Wild plum sauce (green)
8	Wild plum sauce (red)
Total	**20**

ROMANIA

№	**Plants:**
1	Basil leaves
2	Beet
3	Black pepper
4	Cabbage
5	Carrot
6	Celery green and root
7	Cherry leaves
8	Corn flour
9	Corn grits
10	Cucumber
11	Dill
12	Flower of elderberry
13	Garlic
14	Lemon
15	Mint leaves
16	Nettles of spontaneous vegetation
17	Onion
18	Onion green (spring)
19	Parsley green
20	Parsley roots
21	Plum dried
22	Red pepper sweet
23	Rice
24	Sage lives
25	Tomatoes
26	Wheat flour
№	**Food:**
1	Borsch
2	Cornmeal mush
3	Herbal dish
4	Nettle sour soup
5	Plum jam
6	Plum jam, HM
7	Traditional bread
Total	**33**

RUSSIAN FEDERATION

№	**Plants:**
1	Buckwheat, CM
2	Carrot, SM
3	Cucumber, SM
4	Cucumber, Yard
5	Dill, SM
6	Garlic green, SM
7	Juniper berries, Yard
8	Onion green, SM
9	Onion green, Yard
10	Onion, Yard
11	Parsley green, SM
12	Parsley green, Yard
13	Pepper, SM
14	Potato, SM
15	Radish, SM
16	Raisins, Yard
17	Tomato, SM
18	Tomato, Yard
19	Turnip, Yard
№	**Food:**
1	Bread kvass, HM
2	Buckwheat porridge, HM
3	Buckwheat porridge, R
4	Kvass, CM
5	Watermelon juice, CM
6	Watermelon juice, HM
7	Mustard oil, HM
8	Okroshka (with kvass), HM
9	Okroshka, R
Total	**28**

TURKEY

№	**Plants:**
1	Apricots, fresh
2	Apricots, fresh, city market
3	Bay Leaf
4	Black sesame
5	Cabbage
6	Cabbage, red
7	Carrot
8	Cherry
9	Chili
10	Coriander
11	Corn grits
12	Cumin
13	Fennel
14	Green bean fermented
15	Green bean fresh
16	Kale leaves
17	Mint
18	Mulberry
19	Olives
20	Oregano
21	Parsley
22	Plums, green, local
23	Poppy seeds
24	Red pepper
25	Saffron
26	Sumac
27	Tomato
28	Tomatoes
№	**Food:**
1	Aubergine puree
2	Black tea
3	Bread, rolls
4	Bulgur pilaf
5	Corba (Soup)
6	Fermented vegetable
7	Fish salad
8	Sautéed pickled green beans
9	Hot puree
10	Humus, food
11	Kale soup
12	Pepper pasta
13	Roasted chestnut
14	Sauce, pomegranate
15	Tahini halva
16	Tahini sesame paste
17	Thick Yoghurt
18	Tomato cereals mush
Total	**46**

UKRAINE

№	Plants:
1	Apple, dry, CM
2	Apple, fresh, CM
3	Apple, fresh, Patskanyovo, SM
4	Apple, fresh, Yard
5	Apple, fresh, Znyatsevo, SM
6	Beans, Chaslivtsi, SM
7	Beans, CM
8	Beans, Yard
9	Beans, Znyatsevo, SM
10	Beet Rakovets’, SM
11	Beet Rysyno, SM
12	Beet, CM
13	Beet, Yard
14	Cabbage, CM
15	Cabbage, Lviv, SM
16	Cabbage, Storozhnytsya, SM
17	Cabbage, Yard
18	Carrot Dobron’, SM
19	Carrot Rysyno, SM
20	Carrot, CM
21	Carrot, Yard
22	Celery Chaslivtsi, SM
23	Celery Dobron’, SM
24	Cucumber, CM
25	Cucumber, Rysyno, SM
26	Cucumber, Rysyno, SM
27	Cucumber, Velyki Luchky, SM
28	Cucumber, Yard
29	Dill Dobron’, SM
30	Dill, CM
31	Dill, Rysyno, SM
32	Dill, Yard
33	Elderberry flowers, Yard
34	Garlic, CM
35	Garlic, Rysyno, SM
36	Garlic, Vynogradovo, SM
37	Grape, fresh, Yard
38	Green (spring) garlic, CM
39	Green (spring) garlic, SM
40	Green (spring) onion, Yard
41	Melon, CM
42	Melon, SM
43	Nettle leaves, Yard
44	Nettle, Yard
45	Onion Dobron’, SM
46	Onion Rysyno, SM
47	Onion Storozhnytsya, SM
48	Onion, CM
49	Parsley, green Dobron’, SM
50	Parsley, green Rysyno, SM

51	Parsley, green, CM
52	Parsley, green, Yard
53	Parsley, root Dobron’, SM
54	Parsley, root Rysyno, SM
55	Pear, dry, CM
56	Pear, fresh, CM
57	Pear, fresh, Yard
58	Plum, dry, CM
59	Plum, fresh, Yard
60	Potato Storozhnytsya, SM
61	Potato Vynogradovo, SM
62	Potato, CM
63	Potato, Yard
64	Red pepper, CM
65	Sorrel Esen’, SM
66	Sorrel Salovka, SM
67	Tomato, Rysyno, SM
68	Tomato, Vynogradovo, SM
69	Tomato, Yard
70	Yellow-red pepper, SM

№	**Food:**
1	Drevlyanskyy kvass
2	Green Borsch (with sorrel), HM
3	Green Borsch (with sorrel), R
4	Kvass “Yarylo”
5	Lvivskyy kvass
6	Okroshka (with kefir), R
7	Okroshka, HM
8	Pomazanka (with dill), HM
9	Pomazanka (with dill), R
10	Red Borsch (with beet), HM
11	Red Borsch (with beet), R
12	Roasted sunflower seeds, CM
13	Roasted sunflower seeds, HM
14	Uzvar, HM
15	Uzvar, R
Total	**85**

Total number of plants = 169 samples

Total number of traditional foods = 63 samples

Total number of samples: 232 samples

Annex 2. *List of dominantspecies of microorganisms isolated from the plant/food samples*

№	**Species/Genera**
1	*Acinetobacter baumannii*
2	*Actinomyces israeli*
3	*Aerococcus viridans*
4	*Aspergillus carbonaris*
5	*Aspergillus flavus*
6	*Aspergillus nidulans*
7	*Bacillus brevis*
8	*Bacillus cereus*
9	*Bacillus licheniformis*
10	*Bacillus megaterium*
11	*Bacillus pumilus*
12	*Bacillus subtilis*
13	*Bacillus thuringiensis*
14	*Bifidobacterium dentium*
15	*Bifidobacterium longum*
16	*Bacteroides spp.*
17	*Candida albicans*
18	*Candida famata*
19	*Candida membranifaciens*
20	*Candida pelliculosa*
21	*Citrobacter freundii*
22	*Corynebacterium fascians / Arcanobacterium spp.*
23	*Corynebacterium tumefaciens*
24	*Cryptococcus humicola*
25	*Cryptococcus neoformans*
26	*Eikenella corrodens*
27	*Enterobacter aerogenes*
28	*Enterobacter asburiae*
29	*Enterobacter cloacae*
30	*Enterobacter sakazakii*
31	*Enterococcus faecalis*
32	*Enterococcus faecium*
33	*Erwinia herbicola*

34 *Escherichia coli*
35 *Escherichia coli lactose +*
36 *Escherichiacoli lactose-*
37 *Escherichia hermanii*
38 *Eubacterium lentum*
39 *Eubacterium tortuosum*
40 *Klebsiella oxytoca*
41 *Klebsiella ozaenae*
42 *Klebsiella pneumoniae*
43 *Lactobacillus acidophilus*
44 *Lactobacillus casei*
45 *Lactobacillus catenaforme*
46 *Lactobacillus delbrueckii*
47 *Lactobacillus fermentum*
48 *Lactobacillus gallinarum*
49 *Lactococcuslactis*
50 *Lactobacillus mesenteroides*
51 *Lactobacillus plantarum*
52 *Lactobacillus rhamnosus*
53 *Listeria monocytogenes*
54 *Micrococcus luteus*
55 *Neisseria spp.*
56 *Paecilomyces lilacinus*
57 *Pantoea agglomerans*
58 *Pediococcus pentosaceus*
59 *Pediococcus cerevisiae*
60 *Pediococcus damnosus*
61 *Proteus mirabilis*
62 *Proteus vulgaris*
63 *Providencia alcalifaciens*
64 *Pseudomonas fluorescens*
65 *Rhizopus spp.*
66 *Rhodococcus equi*
67 *Saccharomyces cerevisiae*
68 *Salmonella enteritidis*
69 *Sarcina flava*

70 *Scopulariopsis candida*
71 *Serratia ficaria*
72 *Serratia marcescens*
73 *Serratia odoriferabiogroup 1*
74 *Shigella flexneri*
75 *Shigella flexneri (ABC)*
76 *Sporothrix cyanescens*
77 *Sporothrix schenckii*
78 *Staphylococcus aureus*
79 *Staphylococcus auricularis*
80 *Staphylococcus cohnii*
81 *Staphylococcus epidermidis*
82 *Staphylococcus intermedius*
83 *Staphylococcus saprophyticus*
84 *Streptococcus acidominimus*
85 *Streptococcus agalactiae*
86 *Streptococcus lactis*
87 *Streptococcus parauberis*
88 *Streptococcus viridans*
89 *Trichosporon spp.*
90 *Xanthomonas campestris*
91 *Zygosporium masonii*