Studies on Isolation and Identification of Bacteria in Lactic Milk

Agriculture

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Abstract

This scientific work is devoted to the milk produced by cattle farms - cows in Kosovo. Kosovo is a new country and as such is not yet sufficiently consolidated in all its areas of social - political, legal, and economic, health, food and agriculture. Its boundaries are not completely controlled, and it makes for penetrating foreign goods from countries of the region and the world in general. Good of dubious origin food plant or animal, which pose a serious threat primarily to public health and food security in Kosovo market. Control over food quality sound and not be absent for our customers, who do not lose faith in local food products, including dairy products. The purpose of this paper is to understand how the system of technology works and food biotechnology, and focus on driving study elements technological processes of various products and by-products of milk.

Introduction

This scientific paper aims to isolate and identify the lactic bacteria in milk.

As known, milk is the main food for sustaining life and the preservation of human health. From milk derivatives some products, such as cheese, yogurt, cottage cheese, toluene, buttermilk, etc., which are also very important for human existence. Since until now has been written little or nothing from the microbiological aspect, by means of this scientific work we have researched and clarified regarding the notification about the identification and isolation of bacteria that may be in milk and its products.

Milk is a white liquid produced by the glands of milk mammals. It provides the main source of food for all young mammals before they are able to digest other types of food. Breast milk carries with antibodies that affect the immunity of the baby. Milk contains all the necessary nutrients like carbohydrates (lactose), fat, protein, minerals and vitamins (C and B complex).

The main components of milk are: water that constitutes most of his around 85.5-89.5%, 13% fats, proteins 3.4%, 4.8% lactose and 0.8% mineral salts. Milk contains all four phases: the real solution is part of the water, lactose and mineral salts, colloidal phase that includes proteins with water, fat emulsion in water and finally suspension that includes the remnants of microorganisms.

If bacteria are separated in circumstances and in unusual places, then this may suggest that a disease outbreak has occurred (egg Segregation from various equipment and medical instruments atthe hospital),or for deficiencies in aseptic techniques hospital personnel. Classical methods ofidentification of bacteria are based on morphological and metabolic characteristics of them. Selection of diagnostic tests is done by discriminating various. Forpathogen that wewant to identify exit diagnostic test. Already, in microbiological laboratories are more and more on using the molecular biology techniques for the characterization of specific genes.

Material and methods

Tools and material used in the laboratory:

- Evaporator nitrogen, TenDrib locks DB - 3D, at a temperature of 500 C and vortex HeidolphRefax ball,
- Automatic Pipette 20-200 l in, Finn pipette, automatic pipette 100-1000 l in, Finn pipette,
- pipette 20-300 l in automatic multichannel, Fine Pete,
- Plastic 50 ml centrifuge glass civet for evaporation of 15 ml.

For the preparation of samples of raw milk I used these reagents:

- Ethyl acetate acid production by the manufacturer Merck, Germany, is-octane, manufacturer Merck, Germany
- chloroform, manufacturer Merck, Germany, mixture of is-octane and chloroform in the ratio 2: 3 V / V,
- hydrochloric acid 0.1 mol / dm3, manufacturer Merck, Germany,
- PBS has been prepared by digesting the mixture of 0,77g Na2HPO4 + 1,88g KH2PO4 + 8,94gNaClin one liter of distilled water and adjusted to pH of 7.4 0.2, and

• Standard, Sigma-Aldrich manufacturer, where is prepared the basic digestion with the concentrations of 1 mg / ml stored at a temperature of -200C.

They ought this process of dilution by this digestion Figure 1.Is won the standard with a concentration of 10μg / ml. Then, with an additional dilution will be prepared another standard with a concentration of 1000 nag / ml.Finallythe prepared digestion with methanol will be used for the spraying of the milk samples. View from the identification of the milk samples with ordinal numbers.

The laboratory is a place with the necessary tools to conduct research experiments, practical works of scientific, technological or technical, is equipped with measuring instruments or equipment to conduct experiments, research or practice various different sciences. It can also be a class or educational unit.

Its importance, whether in research or industrial scale and in all specialties (Chemical, dimensional, electricity, biology, etc.), lies in the fact that environmental conditions are controlled and standardized, so that it can ensure that are not external impacts (known or anticipated) that change the outcome of the experiment or control.
It ensures that the experiment is being analyzed in that environment, this means that: each laboratory can repeat the process and get the same result, standardization.

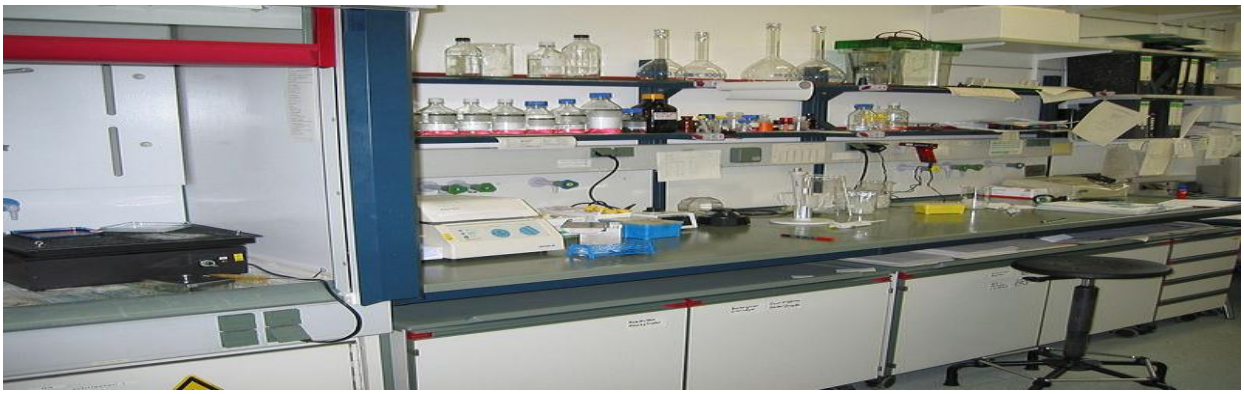


Fig. 1 Analysis of samples taken within and microscopy.


Fig.2. Sampling of milk.

Samples of milk and other food products are cultivated on plate with breeding ground. After incubation for one or several days, can be detected and isolated colonies of bacteria that can be seen with the eye. Each colony contains millions of bacterial cells. During this phase of identification of bacteria the most importancehas the observation of these colonies for the determination of the size, texture, color, andhamlyses (if it is grown on agrarian). Colonies are coloring according by Gram and individual bacterial cells observed with the microscope.

Fig.3. Microscopy of coloniescolored according to Gram.

• Gram stain
Working Methodology
a) Control of purity of glass
b) Preparation of the preparation with bacterial material
c) Drying and fixation of preparation
d) The coloring
a. On the dry preparation wethrow some points from basic coloring solvents, ethylene blue or violet crystal and we leave the dyes to act for 1 min. And we washed it with water for a few seconds.
b. We throw Iodine solution and we leave it to act for 1 min. We washed it with water.
c. We throw alcohol 95% on preparation, pumping point of alkali for 20-30 seconds. It is a critical point of Gram coloring, because if it is long the time of holding on alcohol it can be colored and Gram positive bacteria. Preparation will be washed with water.
d. Take safaris solution and leave it to act 10-20 seconds. Instead of safaris it can be used carbonfuchsine diluted. We wash the preparation with water, dried and look in the microscope with immersion.
Gram positives get blue, while Gram-negative red color.
Sex Lactobacillus and Streptococcus is gram positive. We note in microscope their morphological forms. Sex Lactobacillus is in rod form so bacillus, while sex Streptococcus is the spherical or headaches.
Thetestof catalyze. Throw in plate the peroxide hydrogen 3.5%. Observe for the formation of gas drops. Their presence indicates for the organism'scatalyses positive, and their absence indicates for organism'scatalysesnegative, which should be Lactobacillus and Streptococcus sex.
A colony of bacteria it drains in the glass and further processed as follows:
• Step 1 the colorationwithcrystal violet. Bacteria get blue.
• Step 2 Fixation with iodine. This procedure stabilizes the crystal violet color. Bacteria remain blue.
• Step 3Extraction with alcohol or other solvent. During this procedure some bacteria will be discolored (Gram negative), but others wear the same color (Gram positive).
• Step 4 the coloration withsafaris. Gram positive bacteria remain blue, Gram negative bacteria now they receive the red color of safaris.
On the following will be observed the properties of bacteria with microscope. After the survey, can be observed these questions:

- Are gram-positive or gram-negative the bacteria observed?
- What form they have - rod, headaches, spiral,(variable from)?
- Are they listed separately or are in pairs, chains etc.?
- how large are the observed bacterial cells?

Besides the Gram coloring, in microbiological laboratory practice sometimes are used other types of coloring (coloring as spores and capsules). In the following of the progress of microbiological diagnosis, it will be taken another similar colony of bacteria from the cultivation plates and then will be examined for biochemical properties, such as: does it ferments sugars, such as lactose. In some cases, the bacteria are identified through commercially antibodies that react with defined surface antigens (example by aggregation) .Also for the diagnosis of bacterial infections; in wide use are also many commercial molecular tests. Successful isolation is slow, sometimes even impossible. However, in practice laboratory there is a possibility of detection of bacteria from clinical samples, without cultivation them in nutritional territory.

An example of rapid diagnosis, which is used more in laboratories, is to detect hemolytic streptococcal antigens of group A. This is accomplished by taking smear of product and extracted directly from samples streptococcal antigen (without previously cultivated in nutrient ground.

Results and discussion

Ome of the fields selectively for two of lactic ferments S.thermopiles and L.delbruecki subsp. Bulgariusprovided in tabl.1. Let's make milk test description of the method based on the following work:

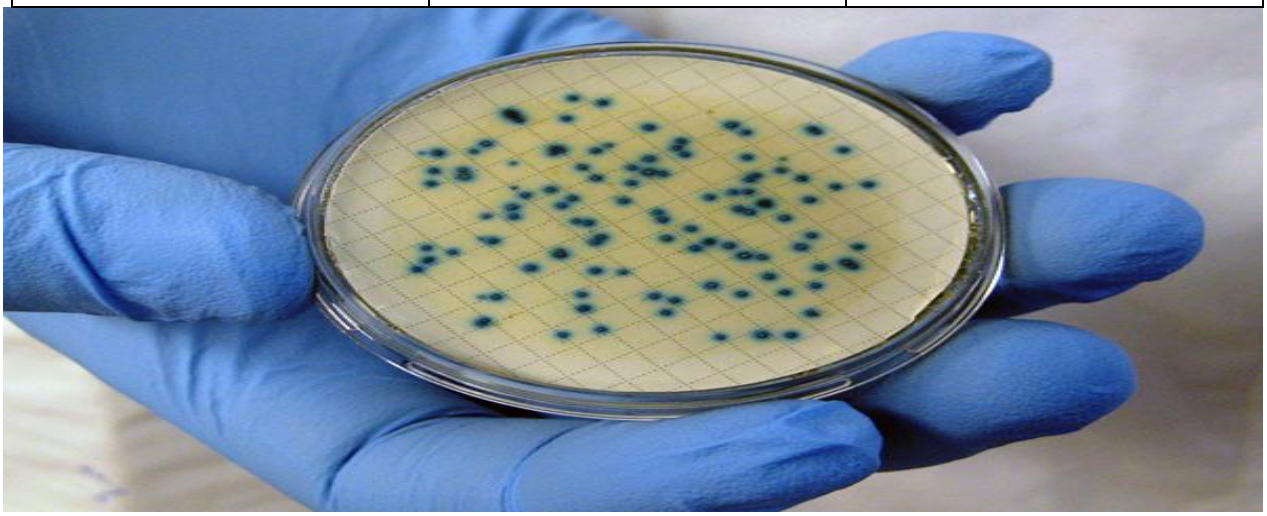
Throw: 25 mml - milk

250 mml water (H2O) of distilled

Take samples 1, 2,3,4,5 and 6 to 9 mml (H2O) and throw in 100 mml (H2O) of distilled in bottles for digestion, then get prepared material and begin the planting of sampling 1.3 and 5 in the type of terrain MRS, where planting is done in an incubator under flame because of the sterilization of the sites of MRS and we begin to spread with the spreader which we sink it in alcohol and spread on the plate of Petri. Then we take the sample and incorporate in autoclaves for 48 h at temperatures of 30 ° C and expect results after 48 h, to make the counting of the colonies. We start first with anisessterilization, take 1 (one) point H2O and decide on the lame, we receive less material (from milk samples) on the plate of Peter and put it on the point of H2O, by spreading as well and so form smear. While the sample material we dry up at the ambient temperature,and then we make the fixing of the flame oflamp 3 times. Now we do the coloring according to program and we put the preparation violet crystals on lamina, and keep 30-60 sec. and we washed it with distilled H2O. Then put iodine for 30-60 sec and again washed it with distilled H2O, the llama sink in alcohol for 10 sec, and again we washed with distilled water. We finally decide sovereign keep for 30-60 sec and sewwashed with distilled water. We dry up with paper and look under the microscope.

Table.1 some of the sites for lactic ferments count

Terrain	*S. thermophilus*	*L.delbrucki* subsp. *Bulgaricus*
TGV agar	Soft colony	Colony with fibrous side
Hansen`s agar	Colony 1-3 mm	Colony 2-10mm
LAB	Soft kolony	Colony irregular, tough
Lee Field	Yellow colony	White colony
Field L-S	Red colony clear area <0.5 mm	Red colony opaque area <1.0 mm
Lactic agar	Not growing	Growing
Agar modified lactic	Small red colony	Colony large white
Tryptose proteose peptone yeast (TPPY) with Eriochrome	Colony 1-3mm oval, with dark center	Colony transparent 4-6 mm, with irregular shape.
TPPY with blue Prussian	Colony blue	Colony white
M17	Mounting	No increase
The acidified MRS	Not Growing	Mounting



Picture. 4. Microscopy

Tab.2. Dairy colonies

Sample	**Average CFU/ml**
Fat	16×10^5
Vita	20×10^5
Primalat	31×10^5
Farm milk one 1	22×10^5
Farm milk two 2	11×10^5
Dukat	53×10^5

Table 2. Reading of milk colonies

In this table is presented counting colonies in the milk, turns out that samples which are listed in the table contain a quantity not limited of lactic bacteria (Enterococos,Leoconostoc,Streptococci etc.) .The obtained results show that of all milk samples analyzed were found at a higher level than the maximum permitted limit for their presence in milk. The table does not present any significant statistical difference between different samples, where the result of the colonies in the milk is close to each of the samples. These organisms are relatedPhenolpeptic to the gender and share the same features with lactobacillus.

- planting is done in agar - blood, Loffler, Tinsel etc.
- OCST - enrichment site that has eggs, cistern, serum and telluride
- Terrain Loffler rich in lipids provides increased rapidly and this field preparations stained with Gram is clear granules Meta chromatic
- Colonies -> 35-37 ° C with or without CO2
- 24 hours colonies are small, it takes 48 hours to grow on blood agar

Identifying Gray colonies-black in cisterns - telluride agar noting typical cells as Chinese characters.

Conclusions

The organization of a scientific paper is to specify all obligations and audited laboratory use fresh milk and processed. Within the realization of my paper my scientific project "Research on the isolation and identification of lactic bacteria in milk," the study was conducted of analytical type - descriptive, assessing each case search and find, set in treatment plan for milk, where are fulfilled the criteria for use of the above mentioned types of milk. Activity of dairies is mainly to produce pasteurized milk, packaged in nylon bags with a shorter time, while the activity of factories, such as "Vita", is primarily concerned with the production of sterilized milk, bottles in cartons. Conditions for the production of milk and putting them into circulation and service and quality assurance of their effectiveness in accordance with relevant legislation implemented in accordance with legal norms, which are permitted and according to HACCP and ISO standards.

References

1. Microbiology of milk and its by-products - from Dr. RozetaHasalliu
2. Dr. ArsimKurti, Assistant Microbiology, Faculty of Medicine
3. Kunz, C; Lonnerdal, B (1990). "Human-milk proteins: analysis of casein and casein subunits ...". American Journal of Clinical Nutrition (American Society for Clinical Nutrition) Retrieved January 14, 2011
4. Walstra P (1979). "Voluminosity of bovine casein micelles and some of its implications." J Sci UK 46.
5. Horne DS (March 1998). "Interactions casein: structure in milk products". Milk Int J.
6. "DP00199: Beta-casein". Taken to March 5, 2012.
7. Fankhauser, David B. (2007). "Fankhauser's Cheese Page" .taken to 09/23/2007. Phosphopeptide-amorphous calcium phosphate. "Journal