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RESEARCH REGARDING THE ROLE OF PROBIOTIC LACTO-ACID PRODUCTS IN INHIBITING THE DEVELOPMENT OF CONTAMINATION MICROORGANISMS

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Abstract

The purpose of our research is to find out the influence the probiotic lacto acid products have in inhibiting the development of some contaminating microorganisms. There were grown the following microorganisms: Escherichia coli, Proteus vulgaris and Bacillus subtilis, in an environment of nutritive agar; after that the probiotic lactic culture was inoculated by means of some sterile microcomprimats (Activia – Danone, Actimel – Danone, Napolife – Napolact, Extra-cream yogurt - Napolact). After impregnating the lactic culture, the samples were at 37ºC for 24 hours, and then there were noticed the development of an area on the microcomprimats, which means that the contaminating microorganisms stopped growing in that area.
***Key words**: probiotic dairy products, acid dairy products, lactic fermentation, intestinal microflora.*

Introduction

Probiotics are defined as aliments or alimentary supplements with living bacteria, which have benefic effects for the human and/or animals, by making the microbial equilibrium from the intestine better. The probiotic dairy products are obtained from milk fermented with specific cultures of lactic bacteria and bifido-bacteria. Now, in this group are gathered a variety of dairy products manufactured at an industrial scale: acid dairy products (yoghurt, bioghurt, buttermilk, kefir, etc.), unfermented milk, sour cream, cheese and ice-cream, different tablets, etc. The acid dairy products obtained through the lactic and lactic plus alcoholic fermentation of lactose represent the most important category.

The lactic fermentation using certain trunks of lacto-bacillus and bifido-bacyeria determines the increase in the milk's nutritional properties, facilitating the digestion and the adjustment of the intestinal functions. It was demonstrated that the fermentation with selective

species of lactic bacteria has numerous favorable consequences on the human's health, which were emphasized in the specialty literature.

The normal equilibrium of the intestinal microflora may be destroyed because of various illnesses, dietary modifications, stress, antibiotic treatment etc., and the consequences being evidently negative for the health. The probiotic products intervene in the moment when the normal equilibrium of the intestinal microflora is destroyed, reestablishing the equilibrium in the intestinal micro flora (Bârzoi, 2002). Nevertheless, the probiotic products have a role in the production of some substances which inhibit the development of pathogenic microorganisms, in stimulating the immune system of the organism, and they also constitute a contribution to the nutritive substances for the organism.

The main species of bacteria used in the manufacturing of probiotic products are: Lactobacillus acidophilus and a variety of Bifido-bacterium species, dominant microorganisms in the human's thin intestine, respectively the thick intestine. Beside these, a series of other lactic bacteria, propionic bacteria and some yeasts, may have positive effect on the health (Costin, 1999).

Among the extremely important probiotic bacteria are Lactobacillus acidophillus and the bifido-bacteria. Lactobacillus acidophillus of a human origin has an antibacterial activity towards an important number of pathogen germs Gram-negative and Gram-positive (Staphylococcus aureus, Listeria monocytogenes, Salmonella tiphymurium, Sighella flexnerei, Escherichia coli, Klebsiella pneumoniae, Bacillus cereus, Pseudomonas aeruginosa, and species of Enterobacter). In contras, Lactobacillus acidophillus does not inhibit the activity of lactobacillus and bifido-bacteria. It was found that the antimicrobial action is independent from the production of lactic acid (Dan, 1999).

The research done in the field of the probiotic dairy products and the probiotic cultures for the human and animals have revealed a series of remarks as: the positive influence on growth, production of riboflavin, niacin, thiamin, vitamin B6, vitamin B12 and folic acid, the intensification in the absorption of minerals, the increase in the immune answer, the reduction in the percentage of pathogen bacteria through the production of lactic and acetic acids, the anti-carcinogenic effects, the rebuilding of the intestinal microflora, the hypo-

cholesterolic effects, and inhibition effect towards the mutagenesis (Saxelin, 1999; Schillinger, 1999).

Experimental

Materials: The inoculum represented by the bacterial culture Escherichia coli, Proteus vulgaris and Bacillus subtilis. The gathering is done from the pure bacterial culture which is homogenized in 10 ml of sterile physiologic serum. The probiotic lactic culture represented by: Activia (with Bifidus essensis), Actimel (with Lactobacillus from the imunitass class), Napolife (contains lactic active ferments: active bifido-bacteria), Yoghurt extra-cream (contains lactic active ferments: active bifido-bacteria). The sterile standard micro-pills are impregnated in the probiotic lactic culture

Experimental protocol: The experiment contains two stages. *In the first stage* there were examined the general effects of the probiotic lacto-acid products on some contaminating germs. *In the second stage,* it was determined the efficiency of the probiotic lacto-acid products, which existed in the market, in inhibiting the development of contaminating microorganisms: the last dilution at which the inhibiting effect appears (Dan, 1997; Bularda, 2000).

The testing of the antibacterial activity of the probiotic lacto-acid products towards the contaminating microorganisms was done through the diffusion-metric method, using micro-pills impregnated in the probiotic lactic culture (Oprean, 2002). The probiotic lactic culture that was put to the test is represented by the following lacto-acid products which exist on the market: Activia (with Bifidus essensis), Actimel (with Lactobacillus casei imunitass), Napolife (containing lactic active ferments: active bifido-bacteria), Yoghurt extra-cream (containing lactic active ferments: active bifido-bacteria). The micro-pills, which contain a fixed quantity of lactic culture, are put on the surface of plates with usual culture environments: nutritional agar already sowed with the tested microorganism (Escherichia coli, Proteus vulgaris and Bacillus subtilis). The efficiency of the antibacterial capacity of the probiotic lactic cultures is appreciated through measuring the inhibition zones, which develop around the micro-pills.

During the experiment, the following stages were run through:

a) The culture environment liquefied and chilled is poured in the Petri plates in a thick layer of 4mm, after which it is put away to harden on a perfectly plane surface.
b) The insemination is done by the method “in cloth”.

c) With the same Pasteur dropper used at the insemination the excess of suspension from the inoculator.
d) The placement of the micro-pills is done with the help of some sterile clamps, at a distance of 15mm from the plate's margins and at 30mm between them.
e) The plates are thermo stated for 24 hours at 37°C.
f) The reading of the results is done through measuring the zones of inhibition with the help of a graded ruler, which is applied on the bottom of the Petri plate.

In the first phase of the experiment, there were inoculated cultures of contaminating microorganisms in a dilution of 10^{-3} on Petri plates on which there were poured usual culture environment: nutritional agar. For each of the four products, it was inoculated a Petri plate with the following microorganisms: a plate with Escherichia coli, a plate with Proteus vulgaris and a plate with Bacillus subtilis. Therefore, as a total there were inoculated 12 Petri plates. The micro-pills were impregnated with probiotic lactic culture in a pure undiluted state. The culture of contamination microorganisms was inoculated in a quantity of 0.5ml in all the Petri boxes.

In the second part of the experiment, dilutions were done for the probiotic lactic cultures of 10^{-1} and 10^{-2}. The pathogen bacteria culture, inoculated on the Petri plates was in the same quantity of 0.5ml. Because of the fact that in the first stage of the experiment, in the case of Bacillus subtilis for the pure undiluted probiotic culture there were not obtained conclusive results, in the second stage pf the experiment the Petri plates were no longer inoculated with it, for the dilutions of 10^{-1} and 10^{-2} of the probiotic lactic cultures. In addition, because in the case of the10^{-1} dilution for the probiotic lactic culture, there was not observed the formation of the inhibition zone also in the case of Proteus vulgaris, so we renounced at inoculating the Petri plates with it, in the case of the 10^{-2} dilutions of the probiotic lactic cultures.

Results and Discussions

From the photos made in the first stage of the experiment (figures 1, 4, 7 – in annex), it is observed that the clearest zones of inhibition appear in the case of the microbial culture of E. coli, on which were placed micro-pills imbued with the lactic culture of the Napolife and Activia products. Also in the case of the microbial culture of Proteus vulgaris it clearly appears inhibitions zones around the micro-pills imbued with the lactic culture of the Napolife and Yoghurt extra-cream products.

From the photos made in the second stage of the experiment (figures 2, 3, 5, 6, 8, 9 – in annex), it is observed that in the case of the microbial cultures of E. coli, there appear zones of inhibition, even through the micro-pills were imbued in 10^{-1} and 10^{-2} dilutions of the Activia and Napolife products. For the microbial culture, P. vulgaris there are no inhibition zones seen, around the micro-pills imbued with the 10^{-1} and 10^{-2} dilutions of the lactic culture.

Conclusions

From the three microbial cultures tested, the most resistant at the action of the probiotic lactic bacteria is Bacillus subtilis and the most sensitive is Escherichia coli. The most efficient results in inhibiting the microbial cultures of E. coli have the lactic bacteria from the Napolife product. The inhibition mechanism of the microbial cultures of E. coli, by the lactic cultures may be explained through the presence of the immunoglobulins, the lyzozim, the lactoferin and lactenins, substances with an antibacterial effect. Nevertheless, from this experiment we may conclude that lactic bacteria from the probiotic products have certain efficiency the inhibition of the microbial culture of E. coli. Actually, E. coli is the most frequent contaminating microorganism from the lacto-acid products.

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Annex

Photos with Escherichia coli and Proteus vulgaris

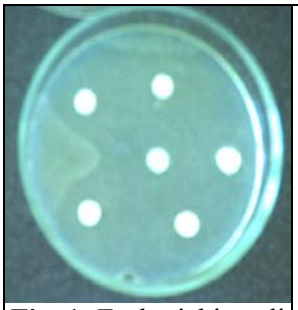

Fig. 1. Escherichia coli – Activia 10^{0}

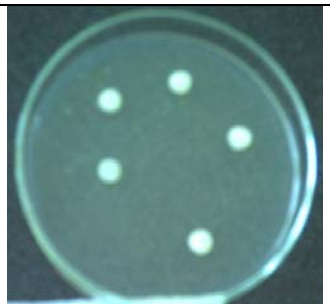

Fig. 2. Escherichia coli – Activia 10^{-1}

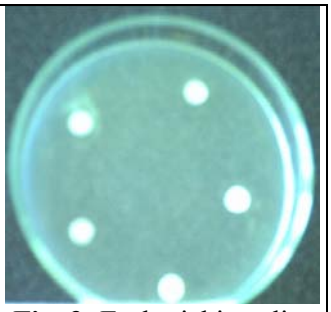

Fig. 3. Escherichia coli – Activia 10^{-2}

Fig. 4. Escherichia coli – Napolife 10^{0}

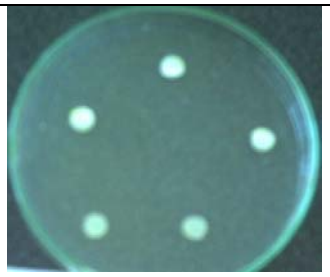

Fig. 5. Escherichia coli – Napolife 10^{-1}

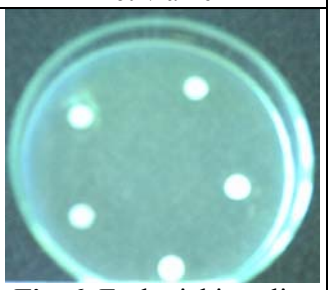

Fig. 6. Escherichia coli – Napolife 10^{-2}

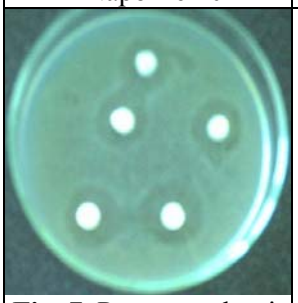

Fig. 7. Proteus vulgaris – Napolife 10^{0}

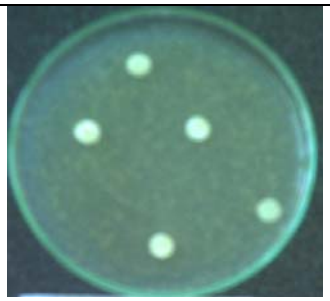

Fig. 8. Proteus vulgaris – Napolife 10^{-1}

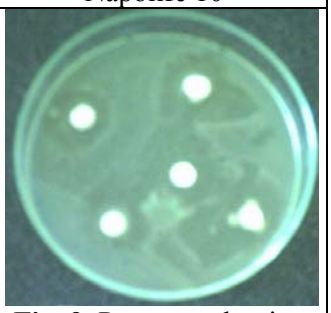

Fig. 9. Proteus vulgaris – Napolife 10^{-2}