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RESEARCH ARTICLE

GROWTH AND CELL VIABILITY IMPROVE OF THE PROBIOTIC STRAIN *LACTOBACILLUS CASEI* SSP. *PARACASEI* IN THE PRESENCE OF OAT BRAN AND BUCKWHEAT FLOUR

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Abstract

The researches on probiotic bacteria in food systems have proved a low viability during preservation, difficulty of their colonization and survival *in vivo*, fact that diminishes the benefic activity on the customers' health. Having as a starting point the above mentioned information, this work presents some research whose main purpose is the improvement of the behavior and functionality of probiotic bacteria *Lactobacillus casei* spp. *paracasei* in fermenting milk by adding oat bran (*Avena sativa*) and buckwheat flour (*Fagopyrum esculentum*), vegetal substrate rich in bioactive compounds. The best results regarding probiotic functionality and stability were obtained by adding 5% and 9% oat bran or buckwheat flour respectively, in fermentative medium. The adding of the vegetal substrate during fermentation increases also the resistance of probiotic bacteria in simulated gastric juice during 90 minutes of incubation.

Key words: *Lactobacillus casei* spp. *paracasei, L. casei* ®431**,** probiotic bacteria, prebiotic vegetal substrate, oat bran (*Avena sativa*), buckwheat flour (*Fagopyrum esculentum*)

Introduction

According to the Food and Agriculture Organization (FAO) and World Health Organization (WHO), probiotics are live microorganisms that give benefits to the consumers when used in adequate amounts as food. They are used mainly in food fermentation such as for fermented milk (Heller, 2001; Mortazavian *et al*., 2006), incorporated into pasteurized milk (Rolfe, 2000), or consumed as live probiotic cells in many nutraceutical products (Ray, 2004; Lavermicocca *et al*., 2005).

Among these, the ones that are considered to be important for healthy living habits are lactobacilli (Elmer *et al*., 2007). *Lactobacillus casei*, which is often found in dairy and meat fermented products has beneficial effects on the immune system, it increases resistance in stressful situations and also diminishes de symptoms of Chron's disease (Itsaranuwat *et al.,* 2003). In order to meet the people's expectation for healthy food, the diversification of fermented food products that contain probiotics and prebiotics is necessary (Michida, 2006).

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For probiotics to render its numerous benefits, they must be able to survive their passage through the human gastrointestinal system. Their tolerance to pH is therefore a critical factor influencing their probiotic functionality (Matto *et al.,* 2006). The low pH of the stomach (pH 1.5 to 2.5) and the antimicrobial action of pepsin are known to provide an effective barrier against the entry of most bacteria into the intestinal system (Bourlioux *et al.,* 2003; Huang and Adams, 2004). Thus, it is important that a study is conducted to evaluate the impact of pH on the viability of the probiotics upon ingestion (Ting and De Costa, 2009).

According to the American Dietetic Association (2008), fibers improve the intestinal functions, reduce blood pressure, can help control weight and improve serum cholesterol levels. Furthermore, the fibers that have prebiotic effects stimulate the bacteria existing in the colon, fact that has a beneficial influence on the health of the host (Gibson, 2004; Guergoletto *et al*., 2010).

Based on these reasons, this work presents some research whose main purpose is the improvement of the functionality and the viability of probiotic bacteria *Lactobacillus casei* ssp. *paracasei* (*L. casei* ®431, a Christian Hansen commercial starter) in fermented milk by adding oat bran (*Avena sativa*) and buckwheat flour (*Fagopyrum esculentum*), rich in bioactive compounds, with prebiotic and protective effects. The tests were conducted *in vitro*, in fermented milk and in simulated gastric juice conditions.

Materials and methods

Chemicals

All chemicals were from Merck KGaA, Darmstadt, Germany.

Probiotic lactic acid bacteria . *Lactobacillus casei* ssp. *paracasei* was provided by Chr. Hansen, Denmark, as freeze-dried commercial starter with commercial name *L. casei* ®431. The storage and maintenance of the culture was carried out as per the recommendation of the manufacturer.

Vegetal substrates. Buckwheat flour (*Fagopyrum esculentum*) and oat bran (*Avena sativa*) were purchased from a market from Republic Moldova and respectively from a Plafar market from Galati, Romania.

Fermented milk sample preparation. Each sample has been obtained from 10 mL inoculum with 11 log Colony Forming Units (CFU)/mL of *L. casei* ®431, 90 mL UHT milk (3.5% fat) in which were added 1% (w/v), 5% (w/v) and 9% (w/v) buckwheat flour or oat bran respectively. A control sample without added vegetal substrate was prepared in the same conditions.

The fermentation was conducted at 37^0C a value of pH 4.6 was reached. The pH was monitored with a pH-meter Portamess ® 911, Switzerland, washed periodically with absolute ethanol to assure the aseptically conditions.

After fermentation, all samples were stored at 4^oC for 21 days.

Probiotic bacteria counting. Viable cell counts were determined by preparing serial decimal dilutions with 0.1% (w/v) peptone water (Merck) which were subsequently plated on MRS agar (Merk) on Petri dishes. The plates were incubated in anaerobic jar (Merck) with Anaerocult® A kit (Merck), for 48 h, at 37°C. Plates containing 25–250 colonies were selected and CFU/mL fermented product was recorded. All plate counts were carried out in duplicates.

Cells viability testing. Simulated gastric juice (SGJ) consisted of 9 g/L of sodium chloride containing 3.0 g/L of pepsin with pH adjusted to 2.0 with hydrochloric acid (Chavari *et al*., 2010).

Since the probiotic fermented milk products have a shelf life of 21 days, it was decided to evaluate the cell viability after an average storage of 14 days.

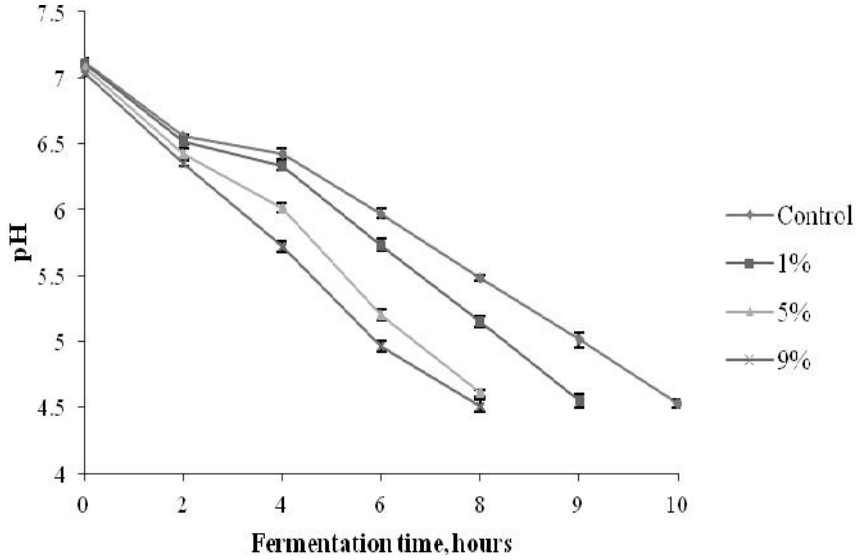
After 14 days of storage, 0.2 mL fermented milk have been taken and homogenized with 10 mL of simulated gastric juice and incubated for 30, 60 and 90 minutes respectively at 37°C. Surviving bacteria were counted by pour plate techniques in MRS agar by anaerobic incubation at 37°C, for 3 days, according to the methods described by Chavari *et al.* (2010). The data is expressed as means from three independent experiments with two replicates.

Results and discussions

Biotechnological behaviour of L. casei ®431 in fermented milk supplemented with buckwheat flour (Fagopyrum esculentum) and oat bran (Avena sativa)

The purpose of the study was to testing the effect of vegetal substrates, in concentrations of 1%, 5% and 9% of buckwheat flour and oat bran, on the growth dynamics and viability of *L. casei* ®431 during milk fermentation. The pH variation and cell multiplication were evaluated. The fermentation was carried under anaerotolerant conditions at 37ºC and stopped for all samples when a pH value of 4.6 was reached.

As showed in Figure 1, the fermentation time needed to reach the pH value corresponding to fermented dairy products varies with the added amount of prebiotics.



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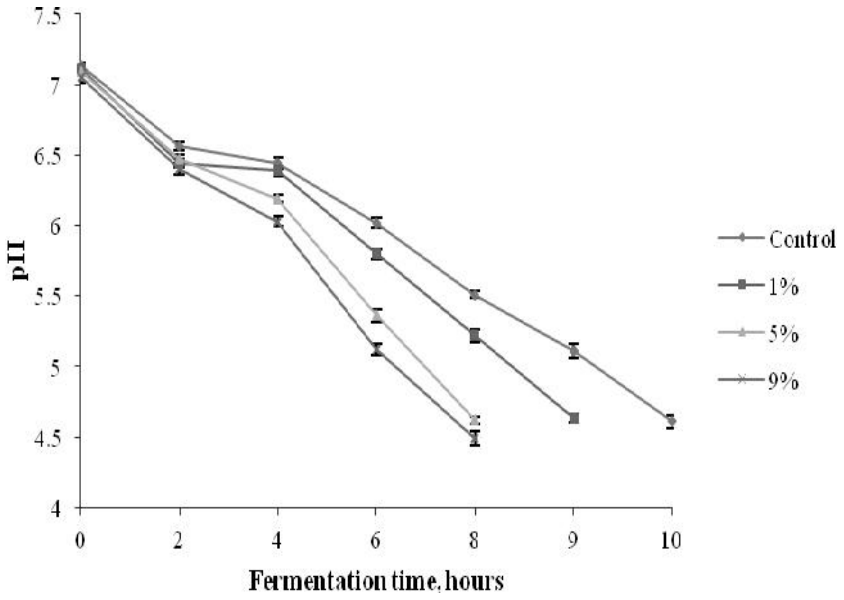

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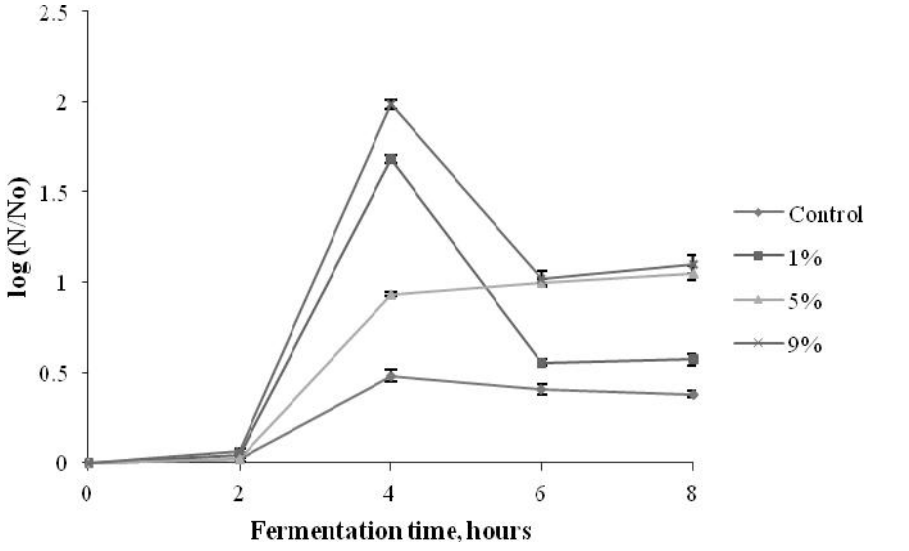
Figure 1. *The evolution of the pH during milk fermentation with L. casei ®431 in presence of different concentrations of buckwheat flour (a) or oat bran (b)*



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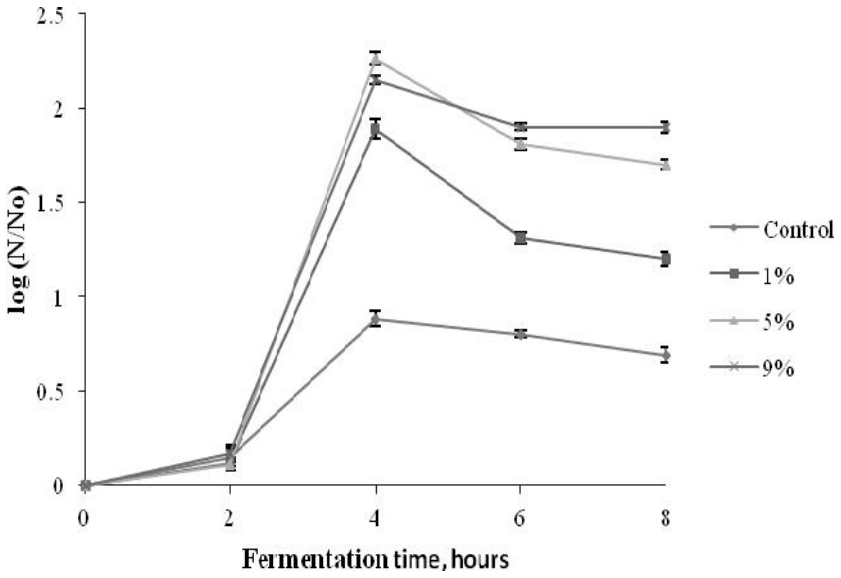

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Figure 2. *The influence of added buckwheat flour (a) or oat bran (b) upon the multiplication rate of L. casei ®431 during milk fermentation*

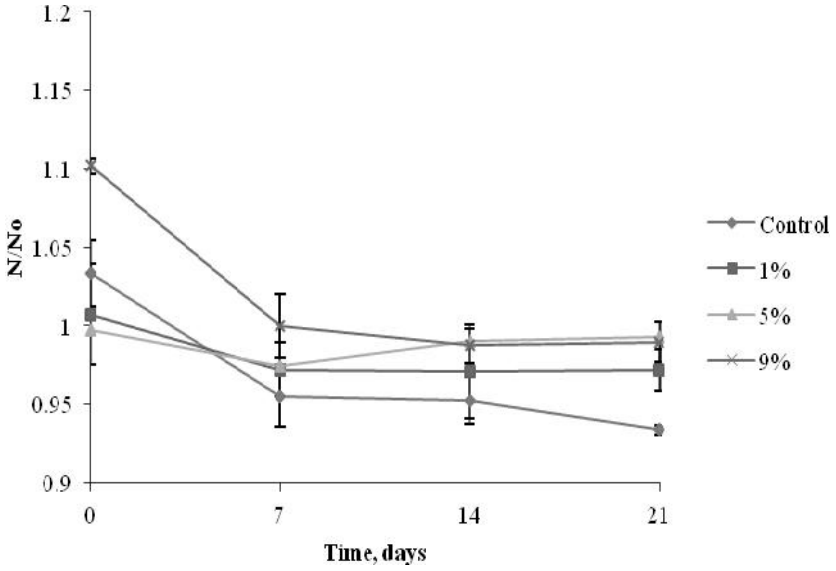
The addition of different concentrations of buckwheat flour and oat bran determines modifications in the fermentation kinetics by shortening the time the pH reaches 4.6 compared to control. The fermentation period decreased, by increasing the concentration of the vegetal substrates, both for buckwheat flour and oat bran in fermented milk. The greatest effect was observed by adding of 5% or 9% vegetal substrates, when the time for pH decrease at value 4.6 was 8 h as compared to the control in which the pH reduction was more slowly.

Multiplication dynamics in the control and fermented milks with added vegetal substrates are presented in Figure 2. The best results were obtained by cultivating *L. casei* ®431 at 37^0C, in UHT milk supplemented with both oat bran and buckwheat flour, by stimulating the multiplication rate, after 4 hours, comparing with the control sample. The multiplication rate is significantly high, 1.5 log CFU/mL compared to control, for the sample with 9% buckwheat flour and 1.4 log

CFU/mL respectively for the sample with 5% oat bran. The use of vegetal substrates as natural additives in fermented milk increased the concentration of probiotic cells by fast multiplication and substantially reduced the fermentation time with 2 h, comparing with the control.

Survival of L. casei ®431 during storage at 4°C in fermented milk supplemented with vegetal substrates

To have probiotic effect, the probiotic strains have to be present in the final product in a sufficiently high number. The probiotic strains have to be ingested at least 6 log CFU/mL to 7 log CFU/mL, which means that in a 100 mL probiotic product portion must be present a number between 8 log CFU/mL or 9 log CFU/mL.



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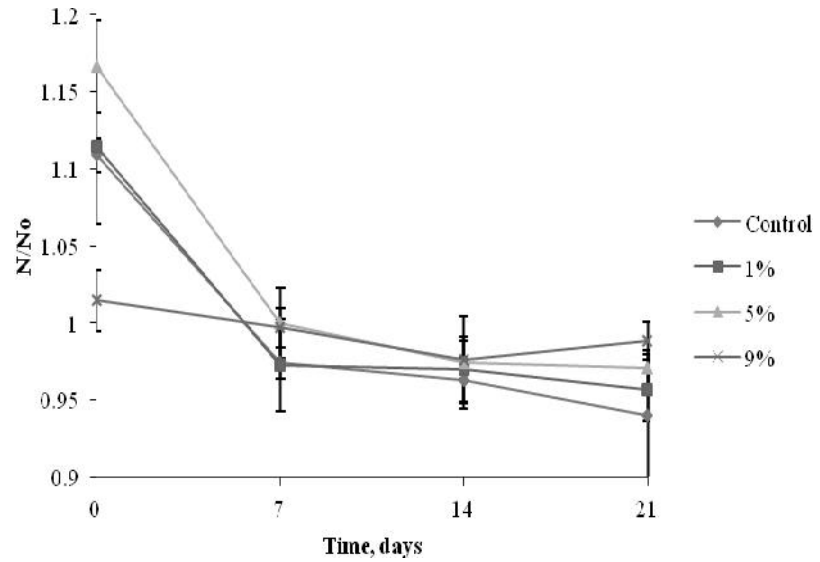

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Figure 3. *Enhance of the L. casei ®431 viability in fermented milk with added buckwheat flour (a) or oat bran (b), during storage at 4°C*

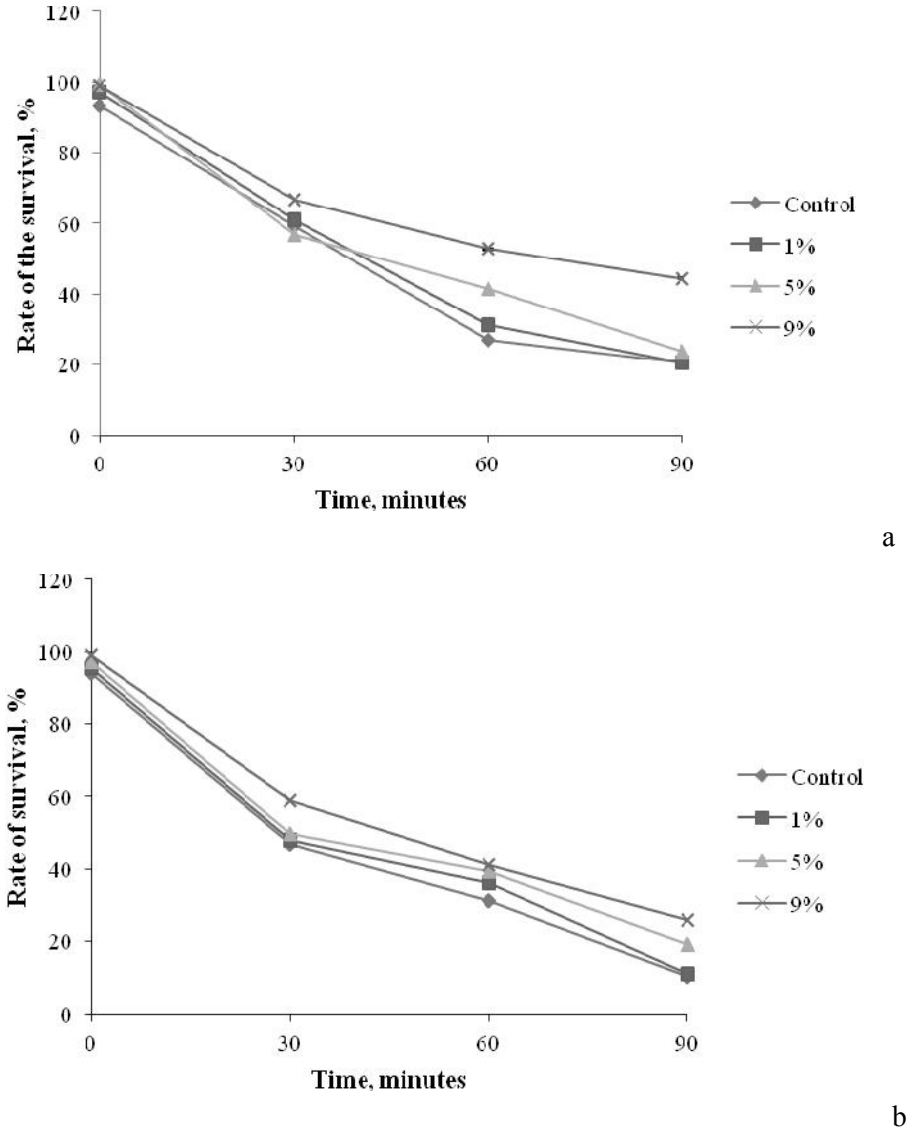

Figure 4. *Rate of the survival in simulated gastric juice of L. casei ®431 grown in milk supplemented with different concentrations of buckwheat flour (a) or oat bran (b)*

Figure 3 show the survival rate of *L. casei* ®431 in fermented milk supplemented with vegetal substrates, during 21 days of storage at 4ºC. In this experiment the start point of evaluation was considered the end of the fermentation time, after 8 h of incubation.

The changes in the survival rate of *L. casei* ®431 were the reference for the survival rate over 21 days as it is considered the shelf life of dairy products (Desai *et al*., 2004).

The logarithmic reduction of cell viability of *L. casei* ®431 at the end of the refrigeration period was similar in the fermented milks with added of buckwheat flour and oat bran respectively, both at 9% concentration.

This suggested that the tested prebiotics in concentration varying between 5% and 9%, enhance the survival of *L. casei* ®431 in the fermented products, during storage at 4°C.

In all cases, the probiotics viability has registered a low decrease after 7 days. The protective effect of vegetal substrates is visible after 14 days and 21 days, in correlation with the concentration of prebiotics.

These results show that after controlling other factors affecting the growth and survival of *L. casei* ®431 such as acid production during fermentation, oxygen content in the product, the antimicrobial substrates produced by lactic acid bacteria (Shah, 2000a and 2000b), the storage temperature (Mortazavian *et al*., 2007), the addition of oat bran and buckwheat flour in concentration of 5% to 9% can have a positive effect on the growth and survival of *L. casei* ®431 in dairy fermented products, preserved in refrigeration conditions.

Evaluation of survival in simulated gastric juice of L. casei ®431 grown in media with prebiotic vegetal substrates

Taking into account the results previously presented, the survival in simulated gastric juice of the *L. casei* ®43, after 14 days of storage of fermented products has been analyzed.

At pH 2.0 at 37^{o}C, the number of viable cells in control decreased with 8 log CFU/mL after 90 minutes of incubation in simulated gastric juice, while in samples with vegetal substrates the reduction was with 4 log CFU/mL (Figure 4).

The best protective effect of the vegetal substrate on the *L. casei* ®431 cells against the acid pH of the simulated gastric juice was highlighted in the 9% samples with both oat bran and buckwheat flour, after 90 minutes of incubation.

The result confirms the positive effect of buckwheat flour and oat bran upon metabolic functionality and stability of *L. casei* ®431 *in vitro* and *in vivo* simulated conditions. Our results are in good agreement with those reported by Guergoletto *et al.* (2010).

Conclusions

The development of new probiotic foods should consider not only the intrinsic characteristics of effective bacterial strains but also the ability of the food matrix to protect the bacterial cells all the way through the gastrointestinal tract.

These results are preliminary and demonstrate that the oat bran and the buckwheat flour can be stimulative and protective additives that are rich in bioactive compounds added in fermented probiotic milks to improve physiologic properties of the probiotic strain, *Lactobacillus casei* ssp. *paracasei*.

To establish the positive effects of prebiotic upon metabolic activity and stability of probiotics an optimization of studied vegetal substrates concentrations will be considered in the next research steps.

Acknowledgements

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