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# SURVIVAL RATE OF CHOSEN *LACTOBACILLUS* BACTERIA TYPE IN MEDIA OF DIFFERENT pH

Kamila Goderska, Maria Czarnecka, Zbigniew Czarnecki

*Department of Fermentation Technology and Biosynthesis, Agricultural University of Poznań, Poland*

ABSTRACT
INTRODUCTION
MATERIALS AND METHODS
RESULTS
CONCLUSIONS
REFERENCES

## ABSTRACT

This work presents the survival rate of the *Lactobacillus rhamnosus* and *Lactobacillus plantarum* bacteria in a changing pH medium inhibiting their growth. The bacteria were kept in pH ranging from 2 – 6, which is a pH of pickled vegetables or stomach. The growth of the bacteria was measured with the use of the method of inoculation onto Petrie's plates and nephelometric method. The longest survival rate of the bacteria was noticed in the pH ranging from 4 – 6. Moreover, the growth of the bacteria is correlated with the absorbance level measured with a nephelometric method. The results of the experiments upheld the presumption saying that the *Loctobacillus plantarum* bacteria are able to survive in man's stomach the time they spend there together with the food.

**Key words:** probiotics, *Lactobacillus plantarum*, *Lactobacillus acidophilus*

## INTRODUCTION

In 21c. a thesis that "nutrition should be an elementary factor extending psycho-physical condition and satisfaction of man's life" becomes more and more important. The above aim is achieved in many ways. One of them is the use of robotic bacteria in the production of food of increased nutritional value, high therapeutic quality and desired sensory characteristics.

A large number of experiments conducted all over the world lengthened the list of “useful micro organism” used in production of robotics, which were originally employed in nutrition of animals and now are also included in man’s diet.

Dairy products based on lactic fermentation bacteria characteristic for their robotic features are currently easily accessible on the market. The most popular today are the dairy products with *Lactobacillus acidophilus* and *Bifidobacterium bifidum* bacteria, which permanently reside in man’s alimentary canal.

We also observe a growing interest in fermented vegetables and seeds in a form of salads or juices, which use in the process of their production robotic bacterial strains.

Probiotic bacteria are characteristic for many antagonistic features when we compare them with gram-positive and gram-negative bacteria, including pathogenetic bacteria.

The mechanism of this process is based on the bacterial competition in the process of gaining the adherence place to the alimentary canal epithelium, stimulation of the organism’s immunity and production of bactericidal substances. [1, 3, 12]. So called non-specific inhibition of the development of pathogens is caused mainly by lactic acid, acetic acid, hydrogen peroxide and bactericides.

Lactic acid bacteria and the products which contain them reveal anti-carcinogenic influence. Feeding a mouse with a yogurt for a relatively short period of time, that is 1-2 weeks, significantly reduced a development of tumors [8]. Those cancericidal features stem mainly from the ability of certain lactic acid bacterial strains to inhibid the growth of feacal microflora of *Clostridium, Staphylococcus* and *Peptostreptococcus* type – producing so called feacal enzymes: glucoronidases, nitroreductases and azoreductases. These enzymes can transform pro-carcinogenic cells into cancerogens. *Lactobacillus acidophilus* used in a diet decreased 2,4 times the activity of β-glucuronidase. Moreover, the living cells of *Lactobacillus acidophilus, Lactobacillus casei* and *Lactobacillus dalbrueckii subsp. bulgaricus* significantly increased the ability of macrophages to execute the process of fagocytosis of carcinogenic cells. Nitrates and nitrites consumed in the process of nutrition can be transformed in intestines into carcinogenic nitrosamines.

Cell absorption of the nitrites by lactic fermentation bacteria reduces the production of nitrosamines [9]. Also *Bifid bacterium longum* bacteria and prebiotics (a group of nutrition elements, which are not digested in the alimentary canal thus stimulating their growth [14]) play an important role in colon tumor prevention.

Lactic acid bacteria are also characteristic for anti-cholesterol features which was proven by Fukushima and Nakano [5] in a series of experiments they conducted on mice. *Lactobacillus acidophilus* and *Streptococcus faecalis* bacteria which bind cholesterol contributed to decreasing its level. A number of bacterial strains were chosen, which were able to assimilate cholesterol in oxygen-free conditions in the presence of gall acids. Moreover, a considerable differentiation of this feature among different *Lactobacillus acidophilus* bacterial strains was demonstrated. Similar features are also revealed by some bacterial strains of *Bifidobacterum sp.* and *Lactobacillus plantarum* [4].

There is a problem, however, concerning the use of lactic acid bacteria in nutrition as many people don't tolerate lactose. So called "lactose intolerance" is characteristic for the deficiency of β-galactosidase which is responsible for the hydrolyze of lactose into D-glucose and D-galactosis. Lactic bacteria cells present in fermented dairy products can decrease the level of lactose even by 50%. Moreover, robotic bacteria release active β-galactosidase enzyme which hydrolyzes lactose [2, 6, 7].

When dealing with people who do not tolerate the taste of fermented dairy drinks the level of lactose is decreased by drinking sweet acidophilic milk with added *Lactobacillus acidophilus* cultures in an amount of $10^{10}$ cells for 1 ml [8].

Lactic acid bacteria play an important role in the prevention of osteoporosis, decay and reduce allergic reaction. As has been shown by Japanese experiments the increase of $Ca^{+2}$ absorption can be achieved thanks to the bacteria of lactic fermentation. It has been proven that in the case of mouse's artificially created osteoporosis we notice an increase of $Ca^{+2}$ absorption and in consequence an increase of bone's durability, after $Ca^{+2}$ has been added to a whey containing living *Bifid bacterium longum* cells. Among bacteria that can be used in fight against decay we include *Lactobacillus GG* of high bactericidal features. The strain is able to inhabit oral cavity mucous membranes and unlike most of the lactic fermentation bacteria it does not ferment lactose, maltose and saccharose, which is a greatly desired feature in such an environment [10, 13].

The above mentioned features of robotic bacteria can be achieved after having injected living bacteria into man's body. Having in mind the alimentary canal's pH level one of their major features has to be the ability to survive in pH lower than 3.0. Furthermore, it is a key property in the case when fermented vegetables are to be the target application of those bacteria. Therefore the survival rate of chosen robotic bacteria was estimated in the medium of pH ranging from 2 to 6.

## MATERIALS AND METHODS

The research material consisted of pure bacterial cultures of *Lactobacillus plantarum* T-106 in a desiccated form coming from Warmińsko-Mazurski University as well as *Lactobacillus rhamnosus* "PEN," "E/N," "OXY" bacterial strains which are an ingredient of *Lakcid forte* medicine available in drugstores. The following nutrients were used for the above bacteria: MRS Agar and MRS Broth manufactured by Fluka Co. The survival rate of the bacteria in the environment of acid pH was measured through inoculation of those bacteria's inoculum with MRS liquid nutrient in the amount of $10^{10}$ cfu (culture-forming unit) for 1 ml.

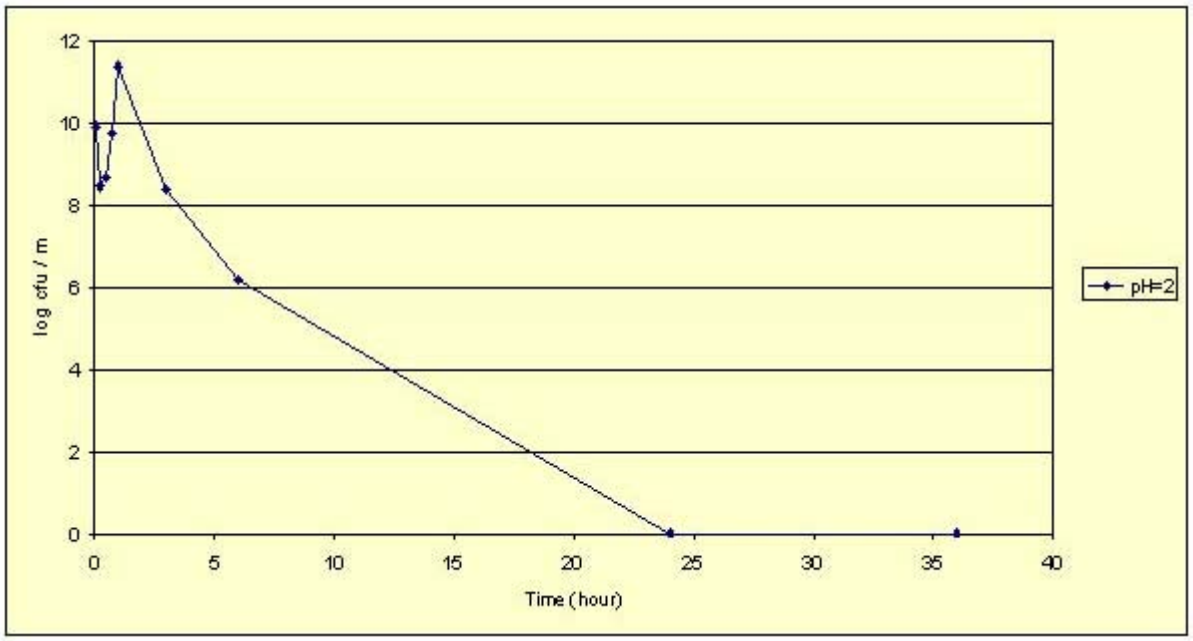
The pH of the environment was regulated with the application of a variable amount (1n) of lactic acid and measured using potentiometric method. Given pH within the range of 2 – 6 was stabilized using Titrisol buffer solution manufactured by Merck Co. The bacterial culture of *Lactobacillus plantarum* T-106 was maintained in the temperature of 30°C and the bacterial culture treated with *Lakcid* nutrient in the temperature of 37°C – both for 48 hours. Then both cultures were cultivated in room temperature for a period of one month. The number of living bacteria was measured using flooding method inoculation onto Petrie's plates. At the same time the number of living bacteria in different pH mediums was measured using nephelometric method employing a wave's length of 660 nm ($\lambda$ = 660 nm).

In order to estimate the influence of the pH of the environment on the survival rate of chosen lactic acid bacteria an analysis of two-factor variances with repetitions was conducted with a use of Excel' 97 spreadsheet.

## RESULTS

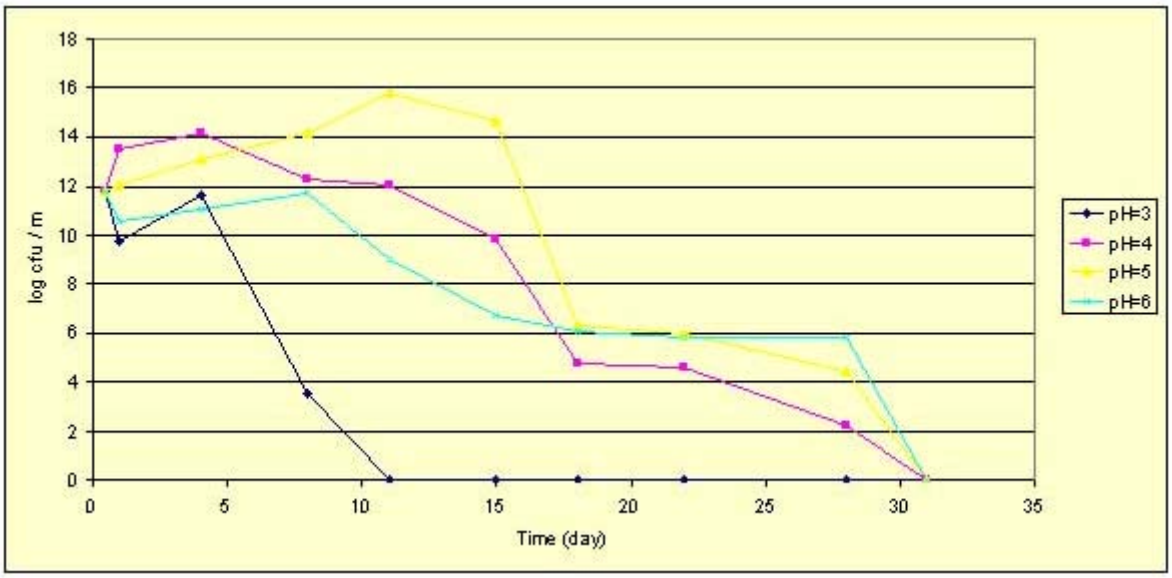
The pH of the environment had a substantial influence on the survival rate of the bacteria of *Lactobacillus plantarum* T-106. In the environment of pH = 2 the number of living bacteria remained at the level of $10^6$ within the first six hours of the experiment. The medium of the pH=2 efficiently inhibited the development of the bacteria and as soon as after 24 hours of the experiment their survival rate came down to 0 (Fig. 1).

**Figure 1. Survival rate of Lactobacillus plantarum bacteria in the medium of pH = 2**




Quite different was the survival rate of the bacteria in the pH ranging from 3 to 6. The number of living bacteria remained at a level of $10^6$ in the medium of the pH = 3 for 4 days of the experiment, in the case of the pH = 4 – for 15 days, in the case of pH equaling 5 and 6 – for 18 days of the experiment. In the last case the time of a total growth cessation was considerably longer. And so for the pH = 3 it equaled 11 days, and for the pH = 4, 5, 6 it prolonged even to 31 days (Fig. 2).

**Figure 2. Survival rate of Lactobacillus plantarum bacteria in the medium of pH = 3, 4, 5 and 6**




A certain pattern can be noticed here, namely, the increase of the number of living bacteria was accompanied by the increase of the level of absorbance estimated using nephelometric method – which manifested itself through the increase of the turbidity of the analyzed media (Table 1).

**Table 1. Absorbance change for Lactobacillus rhamnosus culture in the medium of a different pH**

| Time (days) | Absorbance medium level for the culture in the of pH: | | |
|---|---|---|---|
| | 4 | 5 | 6 |
| 2<br>mean values± SD | 0.066± 0.002 | 1.224± 0.005 | 1.582± 0.008 |
| 6<br>mean values± SD | 0.112± 0.007 | 1.617± 0.008 | 1.766± 0.003 |
| 9<br>mean values± SD | 0.110± 0.003 | 1.611± 0.001 | 1.807± 0.003 |
| 14<br>mean values± SD | 1.006± 0.011 | 1.662± 0.002 | 1.772± 0.010 |
| 17<br>mean values± SD | 1.140± 0.012 | 1.716± 0.053 | 1.772± 0.010 |
| 27<br>mean values± SD | 1.438± 0.002 | 1.716± 0.053 | 1.772± 0.010 |
| 41<br>mean values± SD | 1.438± 0.002 | 1.716± 0.053 | 1.772± 0.010 |

The pH of the environment also had an impact on the survival rate of *Lactobacillus rhamnosus* bacteria. The number of living bacteria of *Lactobacillus rhamnosus* remained at a level of $10^6$ on the substratum of the pH = 2 and 3 for 0,5 hour. The number of living bacteria of lactic type on the substratum of pH = 2 came down to 0 after 1 hour of the experiment and on the substratum of pH = 3 in the ninth hour (Fig. 3).

**Figure 3. Survival rate of Lactobacillus rhamnosus bacteria in the medium of pH = 2, 3**

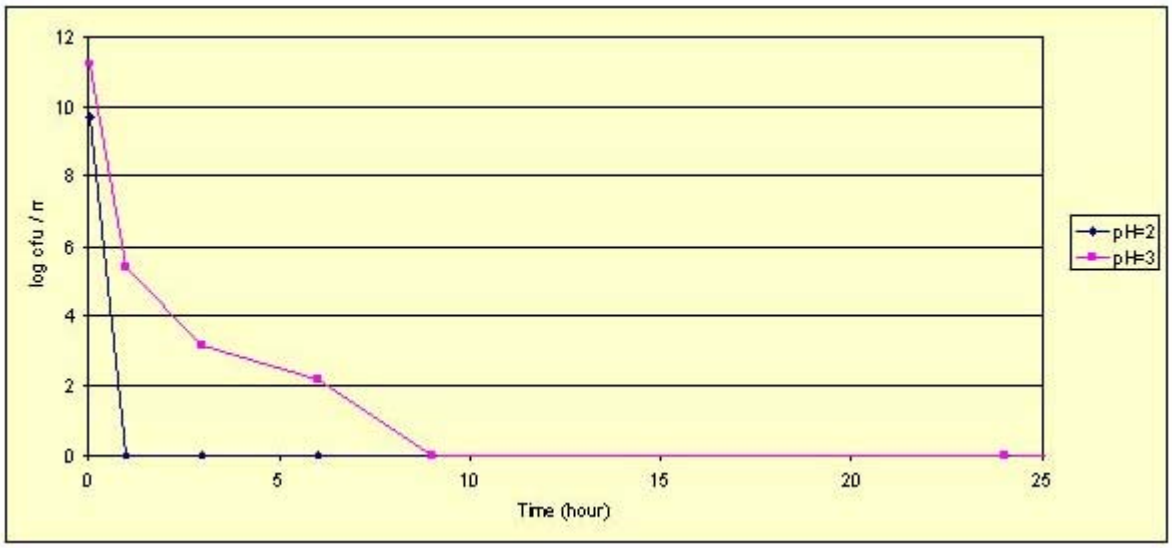
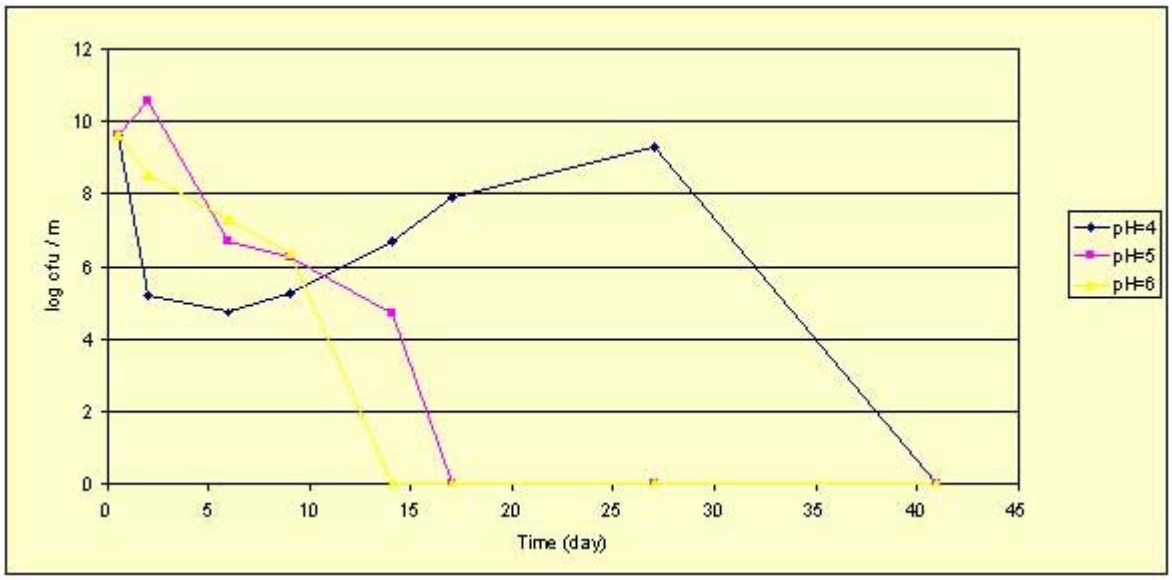



**Figure 4. Survival rate of Lactobacillus rhamnosus bacteria in the medium of pH = 4, 5, 6**




The number of living bacteria on the substratum of pH of 4, 5 and 6 remained at the level of $10^6$ for 9 days of the experiment. The increase of the number of bacteria kept in pH = 4 was continually restricted between the 27$^{th}$ and 41$^{st}$ day of the experiment. The number of living bacteria kept in the medium of pH = 5 and 6 was came down to 0 faster than in the case of the culture kept in the environment of pH = 4. In the case of pH = 5 it happened after 17 days and in the environment of pH = 6 after 14 days (Fig. 4). Analyzing the changes of absorbance during the experiment with *Lactobacillus rhamnosus* cultures on the substratum of pH = 4,5 and 6 one can notice that an increase of the number of living bacteria brings about an increase of absorbance for those cultures (Table 2). Having analyzed the changes of turbidity of the substratum of different acidity levels, inoculated with the bacteria of *Lactobacillus plantarum* T-106 and *Lactobacillus rhamnosus* it has been noticed that the growth of the bacteria is correlated with an increase of the absorbance in medium of pH of 3, 4, 5 and 6. With the decrease of the living microorganisms the absorbance level remains unchanged which is reflected by the lower correlation index (e.g. for *Lactobacillus plantarum* pH = 5 and pH = 6, r respectively = 0.14 and –0.26; for *Lactobacillus rhamnosus* pH= 4, r= 0.48).With the increase of living cells the level of absorbance also increases which is reflected by the higher correlation index (e.g. for *Lactobacillus plantarum* pH = 5 and pH = 6, r respectively = 0.57 and 0.78; for *Lactobacillus rhamnosus* pH = 4; r = 0.65)- (Tables 1 and 2). It remains unchanged, however, at a fixed level when the number of living bacteria in the samples starts coming down to 0.

For that reason the nephelometric method cannot be used as a reliable measure method for estimating the survival rate of the bacteria, although it is beyond a shadow of a doubt that a sudden increase of the sample's turbidity indicates an increase of bacterial cells.

**Table 2. Absorbance change for Lactobacillus plantarum culture in the medium of a different pH**

| Time (days) | Absorbance level for the culture in the medium of pH: | | | |
|---|---|---|---|---|
| | 3 | 4 | 5 | 6 |
| 1<br>mean values± SD | 0.357± 0.008 | 0.843± 0.014 | 1.406± 0.053 | 1.696± 0.062 |
| 4<br>mean values± SD | 0.336± 0.016 | 1.440± 0.002 | 1.670± 0.065 | 1.827± 0.027 |
| 8<br>mean values± SD | 0.397± 0.017 | 1.583± 0.002 | 1.811± 0.021 | 1.864± 0.003 |
| 11<br>mean values± SD | 0.392± 0.029 | 1.623± 0.004 | 1.853± 0.006 | 1.890± 0.013 |
| 15<br>mean values± SD | 0.526± 0.029 | 1.746± 0.014 | 1.931± 0.001 | 1.950± 0.008 |
| 18<br>mean values± SD | 0.460± 0.007 | 1.707± 0.007 | 1.855± 0.014 | 1.949± 0.093 |
| 22<br>mean values± SD | 0.538± 0.031 | 1.849± 0.010 | 2.054± 0.066 | 2.013± 0.093 |
| 28<br>mean values± SD | 0.522± 0.002 | 1.793± 0.010 | 2.054± 0.066 | 2.013± 0.013 |
| 41<br>mean values± SD | 0.522± 0.002 | 1.793± 0.010 | 2.054± 0.066 | 2.013± 0.013 |

## CONCLUSIONS

1. The survival rate of the bacteria of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* T-106 depends on the level of acidity of their substrate. The longest survival rate of 1 month can be noticed in the cultivation medium of pH = 4 – 6.
2. Survival rate of *Lactobacillus plantarum T-106* bacteria in the environment of pH = 2 reaching even 24 hours entitles us to make a conclusion that this type of bacteria is able to survive in man's stomach the period of time they spend there together with food. When it comes to *L. rhamnosus* bacteria this amount of time is slightly shorter.
3. An increase of the number of living bacteria of *Lactobacillus* type brings about an increase of the absorbance level measured using nephelomertic method. Those two increases weakly correlate with one another. The method, however, cannot be used as a reliable measurement method for the estimation of the survival rate of the above bacteria.

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Kamila Goderska, Maria Czarnecka, Zbigniew Czarnecki
Department of Fermentation Technology and Biosynthesis
Agricultural University of Poznań
Wojska Polskiego 31, 60-624 Poznań, Poland
ph. (061)8487279
fax. (061)8487314
e-mail: kamilag@owl.au.poznan.pl



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