

COMPARISON A STORAGE METHODS OF LACTIC ACID BACTERIA

Gulnar T. Dzhakibaeva, Karligash M. Kebekbaeva

“Institute of microbiology and virology” CS MES RK, Kazakhstan, Almaty, Bogenbay-batir str , 103

Abstract

Conducted research on the influence of storage methods on the viability and activity of probiotic lactic acid microorganisms from the collection of the Institute of Microbiology and Virology. It is shown that the tab for storage in many ways not all tested strains retained probiotic activity.

*From ten studied lactic cultures four cultures: *Lactobacillus plantarum* 2, *Lactobacillus cellobiosus* 20, *Lactobacillus curvatus* 18d, *Lactobacillus salivaris* 8d didn't show antagonistic activity in relation to *Bacillus subtilis* at one method storage. From the data obtained follows that storage by method of reseeded a negative impact on the preservation the antimicrobial properties of lactic acid bacteria. At storage of lactic cultures under a layer of vaseline oil and in 10% glycerin solution at low temperatures lactic cultures keep antimicrobial properties.*

The most acceptable methods for the storage of lactic microorganisms is storage under a layer of mineral oil and 10% glycerol solution at low temperatures.

Key words: *viability, activity, probiotic, storage, strains, lactic acid bacteria, microorganisms.*

Thanks to scientific achievements of microbiology, introduction of new development of genetic engineering began possible to receive hi-tech strains of microorganisms, producers of enzymes, amino acids, antimicrobial metabolites and other biologically active agents. Thus the integral component of fundamental base practically any biotechnological project are collections of microorganisms which guarantee preservation of resources of a microbial variety and make them available for use in scientific researches.

The problem of preservation of microbial diversity is as global as questions sustaining the environment. One of the important tasks is to develop a collection of the most effective methods for long-term storage of microbial cultures to ensure long-term preservation of viability and biological characteristics [Gavrilova N., 2003].

Collections of cultures of microorganisms play an important role in the study of microbial diversity and the preservation of the genetic stability of producing biologically substances and microbial mass for practical use in the present and in the future in various sectors of agriculture and medicine, food, processing and other industries.

The special attention is paid to the groups of bacteria representing potential interest as objects of biotechnology and playing an important role in functioning of various ecosystems.

Interest to studying of lactic bacteria is explained by a wide range of their biological activity and harmlessness for the human and animals. Traditional methods of maintaining the lactic acid bacteria are reduced to their growing on rich nutrient media with frequent subculture. Elective medium is skim milk (skim). A bacterium grown in skimmed milk is stored in the form of milk clot within a month. However, frequent periodic subcultures lead to reduced survival of the bacteria, changing the composition of the population, the loss of a series of physiological and biochemical properties.

Heterofermentative lactic acid bacteria may lose the ability to form volatile acids, CO₂, and go to the path of homofermentative fermentation of hexoses. Known methods for the storage of cultures grown in media specific for the bacteria, in 25 % solution of glycerol or a layer of vaselin oil at - 20°C, in a state freeze- dried using a protective medium as cryoprotectants altering the osmotic pressure in the environment and reducing the magnitude of the activity water. [Kvasnikov E. et all. 1976]. As protective media are aqueous solutions of various components. Lyophilization provides long-term viability of lactic acid bacteria. Selection lyophilization conditions and the choice of a specific protective environment for different types of bacteria.

In recent years the special attention of researchers is attracted to pro-biotic properties of these bacteria [Ratnikova I., 2007; Ratnikova I., Gavrilova N., 2009, 2010]. Analysis of the available literature data testifies a multifaceted impact of probiotics on microecology of a digestive tract. One of the way to fight with infectious diseases is the use of a treatment and prophylaxis of lactic acid bacteria and their metabolites. These microorganisms are symbionts gastrointestinal tract, harmless for humans and animals.

The most important aspects of the interaction of probiotic strains with intestinal microflora and the human body and animals are the formation of antibacterial substances competition for nutrients and adhesion places, stimulation of the immune system [Khoroshilova N., 2003. Servin A., 2004. Ammor S., 2006].

It is noted that among the same types meet both strong and weak antagonists that testifies an antagonistic activity is more a strains sign [Tulemisova K., 2004]. The data given above testify to need of maintenance of a collection of active strains of lactic bacteria and replenishment by its new strains with pro-biotic properties.

MATERIALS AND RESEARCH METHODS

Objects of study were 10 strains of lactic acid bacteria from the collection of the laboratory: *Lactobacillus plantarum* № 53H, *Lactobacillus plantarum* 22, *Lactobacillus plantarum* 2, *Lactobacillus cellobiosus* 20, *Lactobacillus acidophilus* 27W, *Lactobacillus curvatus* 18δ, *Lactobacillus casei* 139, *Lactobacillus casei* 173a, *Lactobacillus salivarius* 8δ, and *Lactobacillus fermentum* 27.

As culture media for lactic acid bacteria, the medium MRS.

Medium composition MRS:

1. Peptone 10.0 g
2. Beef extract 10.0 g
3. Yeast extract 5.0 g
4. Dextrose 20.0 g
5. Tween 80 1.0 ml
6. Ammonium citrate 2.0 g
7. Sodium acetate 5.0 g
8. Manganese sulphate 0.05 g
9. Magnesium sulfate 0.1 g
10. Potassium phosphate dibasic 2.0 g
11. Water - 1 liter

Quantity of viable cells of lactic acid bacteria was determined by the turbidity of the same titer for all three methods of storage, then the serial dilution method, followed by plating on agar media grown microcolonies were counted.

Order to determine antagonistic activity of lactic acid bacteria against *E.coli* was used solid medium in the wort agar mixture in a 1:1 MPA.

Antagonist activity installed by the diffusion method against the test cultures: *Escherichia coli*, *Bacillus cereus*.

The studied organism seeded solid "lawn" on the surface of an agar plate in a Petri dish. After the organism grows well, cork drill (diameter about 8 mm) was cut agar blocks are transferred to the other surface of the agar plates, previously inoculated with one of test organism. One Petri dish can be placed 6-7 agar blocks. Plates of agar blocks were placed in an incubator for 20-24 hours at a temperature favorable for the development of the test organism. If the organism secreted by the antibiotic inhibits the development of the test microbe, then around the agar block the zone is formed lack of growth. The more an antibiotic is released or formed by the more active antibiotic substance, the greater the diameter of the zone of non-growth test microbe.

RESULTS AND DISCUSSION

All lactic acid cultures laying in storage in various methods are unique in their properties and didn't lose relevance today. Choice of the methods of storage are individual for each cultures. It must be based on

preservation of viability, morphological characteristics, physiological characteristics during maximum storage time of cultures as well as the simplicity and reliability of the implementation of this method.

With the purpose of selecting the optimal method for preservation of lactic acid bacteria under conditions of prolonged storage collection strains carried laying 10 strains in three ways: by subculturing, storage under a mineral oil, stored in glycerol at low temperatures.

Table 1 - Viability of lactic acid microorganisms under different storage methods

Names of cultures	Viability, KOE, ml		
	Storage on a solid medium	Storage under vaseline oil	Storage in 10% glycerol solution at low T(-20°C)
<i>Lactobacillus plantarum</i> 53H	11 x10 ⁷	10 x10 ⁷	12 x10 ⁷
<i>Lactobacillus plantarum</i> 22	13 x10 ⁵	9 x10 ⁶	5 x10 ⁶
<i>Lactobacillus plantarum</i> 2	10 x10 ⁷	8 x 10 ⁷	9x 10 ⁷
<i>Lactobacillus cellobiosus</i> 20	8 x 10 ⁷	7 x 10 ⁷	7 x 10 ⁷
<i>Lactobacillus acidophilus</i> 27w	3x10 ⁵	3x10 ⁶	10x10 ⁶
<i>Lactobacillus curvatus</i> 18d	8x10 ⁶	4x10 ⁶	8x10 ⁶
<i>Lactobacillus casei</i> 139	10x10 ⁶	7x10 ⁶	9x10 ⁶
<i>Lactobacillus casei</i> 173a	6x 10 ⁶	5x 10 ⁶	8x 10 ⁶
<i>Lactobacillus salivaris</i> 8d	8 x 10 ⁷	9 x 10 ⁷	10 x 10 ⁷
<i>Lactobacillus fermentum</i> 27	6x10 ⁶	5x10 ⁶	8x10 ⁶

From the results presented in Table 1 shows that the best storage method for a lactic acid microorganism was storage under the layer of vaseline oil and 10% glycerol solution at low temperatures (-20C). The survival rate during storage by reseeded for such cultures as L. 2 plantarum, L. curvatus 18, L. salivaris 8d was worse than when stored under a layer of vaseline oil and stored in 10% glycerol solution at low temperatures.

The antimicrobial activity of lactic microorganisms was tested to the test-culture of E.coli (Table 2). All lactic acid bacteria in varying degrees, showed antagonistic activity against E.coli.

The best indicators on antimicrobial activity were obtained after storage of lactic acid cultures in 10% glycerol solution at low temperatures.

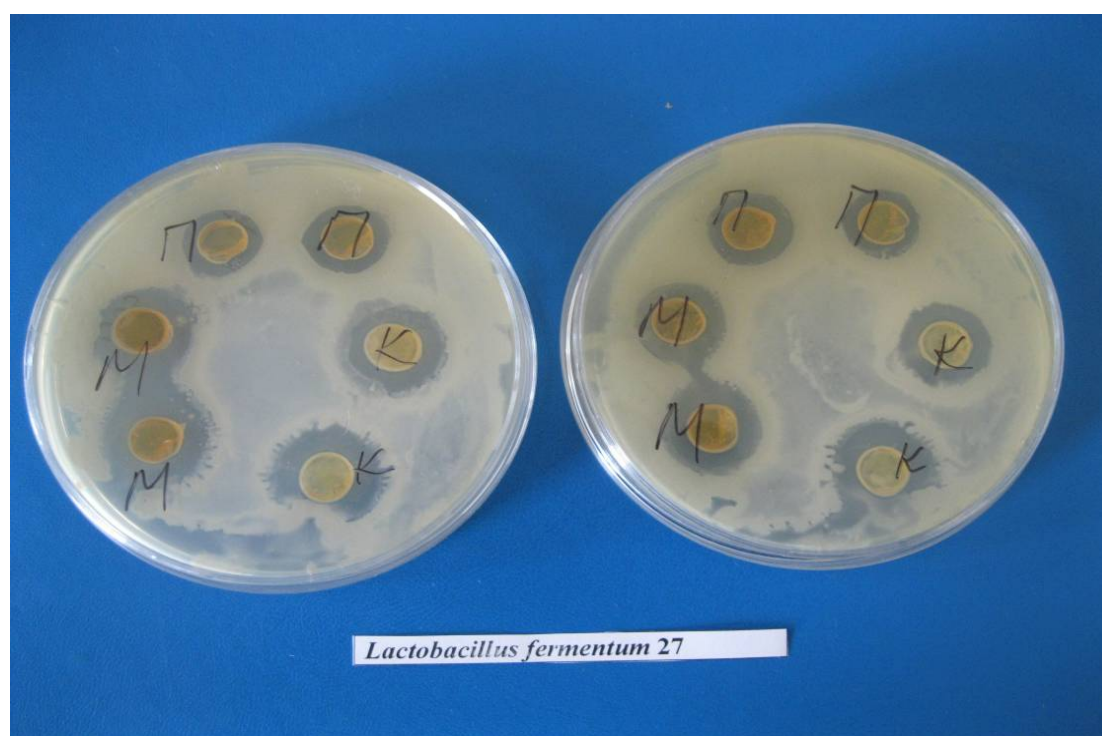
The diameters of the zones of growth suppression in *E.coli* at such cultures as *L.plantarum* 53H, *L.cellobiosus* 20, *L.fermentum* reached the 27 from 15 mm to 20 mm.

Weaker pro-biotic activity was shown by culture of *Lactobacillus casei* 139 at all three methods of storage. At storage by a reseeded method the best antimicrobial activity was shown by cultures - *L. acidophilus* 27w and *L.fermentum* 27.

At L. plantarum 2 after storage by a method of reseedings it wasn't observed antimicrobial activity. At storage of lactic cultures under vaseline oil all cultures showed antimicrobial activity in relation to test culture of E.coli. The best indicators for antagonistic activity were in cultures of L. fermentum 27 at all three storage methods. Thus, after storage of lactic microorganisms such methods as a method of passages, storage under paraffin oil and 10% glycerol solution at low temperatures was saved antibacterial activity of lactic acid microorganisms in the intestinal pathogen diseases - *E.coli*.

Table 2 – The antagonistic activity of lactic acid microorganisms

Names of cultures	The diameters of the zones of growth suppression <i>E.coli</i>		
	Storage on a solid medium	Storage under vaseline oil	Storage in 10% glycerol solution at low T(-20°C)
<i>Lactobacillus plantarum</i> 53H	12±0,1	15,5±0,2	14±0,4
<i>Lactobacillus plantarum</i> 22	10±0,1	13,5±0,6	13±0,7
<i>Lactobacillus plantarum</i> 2	0	12±0,2	15±0,1
<i>Lactobacillus cellobiosus</i> 20	11±0,4	13,5±0,5	20±0,3
<i>Lactobacillus acidophilus</i> 27w	13,7±0,5	13±0,6	10±0,1
<i>Lactobacillus curvatus</i> 18д	13±0,6	12±0,4	13±0,6
<i>Lactobacillus casei</i> 139	10±0,5	11±0,2	10±0,2
<i>Lactobacillus casei</i> 173a	11±0,1	11±0,3	13±0,4
<i>Lactobacillus salivaris</i> 8д	12,5±0,5	13±0,3	15±0,1
<i>Lactobacillus fermentum</i> 27	15±0,1	17±0,5	18±0,5



П- method of reseeding, M-storage under vaselin oil, K-storage in 10% glycerol solution at low temperatures T(-20°C)

Figure 1– An antagonistic activity of *L. fermentum* 27 to test-culture of *E.coli*

Researched the antibiotic properties of lactic acid microorganisms to the test culture, causing intestinal infection *Bac.subtilis* (Table 3). During storage of lactic acid cultures o on a solid medium by passages neither of the

cultures didn't show antagonist activity at seeding on MRS agar medium except the culture *L. plantarum* 53H. At storage of lactic cultures under a layer of vaseline oil on a slanted agar five cultures showed antagonistic activity; from them four cultures: *L. plantarum* 22, *L. acidophilus* 27w, *L. casei* 139, *L. casei* 173a showed the increased antimicrobial activity. Storage in 10% glycerol solution at low temperature gave a similar pattern that during storage under vaseline oil; five cultures showed an increased antimicrobial activity.

Table 3- An antagonistic activity of lactic acid bacteria

Name of cultures	The diameters of the zones of growth suppression <i>Bac.subtilis</i> , mm		
	Storage on a solid medium	Storage under vaseline oil	Storage in 10% glycerol solution at low T(-20°C)
<i>Lactobacillus plantarum</i> 53H	15±0,7	14±0,3	15±0,4
<i>Lactobacillus plantarum</i> 22	0	20±0,1	22±0,3
<i>Lactobacillus plantarum</i> 2	0	0	0
<i>Lactobacillus cellobiosus</i> 20	0	0	0
<i>Lactobacillus acidophilus</i> 27w	0	17±0,9	25±0,3
<i>Lactobacillus curvatus</i> 18д	0	0	0
<i>Lactobacillus casei</i> 139	0	20±0,1	0
<i>Lactobacillus casei</i> 173a	0	20±0,4	20±0,1
<i>Lactobacillus salivaris</i> 8д	0	0	0
<i>Lactobacillus fermentum</i> 27	0	0	22±0,7



II- method of reseeding, M-storage under vaselin oil, K-storage in 10% glycerol solution at low temperatures T(-20°C)

Figure 2 – An antagonistic activity *L. plantarum* 53H to the test-culture *Bac.subtilis*

From ten studied lactic cultures four cultures: *L. plantarum* 2, *L. cellobiosus* 20, *L. curvatus* 18d, *Lactobacillus salivarius* 8d didn't show antagonistic activity in relation to *Bac. subtilis* at one method storage.

From the data obtained follows that storage by method of reseeded a negative impact on the preservation the antimicrobial properties of lactic acid bacteria. At storage of lactic cultures under a layer of vaseline oil and in 10% glycerin solution at low temperatures lactic cultures keep antimicrobial properties.

The conducted researches on the influence of storage methods on the viability and activity of probiotic lactic acid microorganisms have shown that the lay down for storage in many ways all tested strains didn't retain a probiotic activity.

The most acceptable methods for the storage of lactic microorganisms is storage under a layer of mineral oil and 10% glycerol solution at low temperatures.

REFERENCES

Ammor S., Tauveron G., Dufor E., Chevallier I. Antibacterial activity of lactic acid bacteria against spoilage and pathogenic bacteria isolated from the same meat small-scale facility: Screening and characterization of the antibacterial compounds // Food Control. - 2006. - Vol. 17, № 6, - p. 454-461.

Gavrilova N.N., Ratnikova I.A. The main achievements of the laboratory microbial preparations in biotechnology, Izvestiya of NAN RK series of biological and medical sciences. - 2003. - № 2., p. 48-57.

Gavrilova N.N., Ratnikova I.A. Creation of wide spectrum of action of probiotics / / Abstracts of the International Congress "Biotechnology substance and prospects of development". - Moscow, 2010. - p. 471.

Castro M.P., Palavecino N.Z., Herman C., Garro O.A., Campos C.A. Lactic acid bacteria isolated from artisanal dry sausages: Characterization of antibacterial compounds and study of the factors affecting bacteriocin production // Meat Science. - 2001. - Vol. 87, № 4, - p. 321-329.

Khoroshilova N.V. An immunomodulatory and therapeutic effects of probiotics / / Immunology. - 2003. - № 6. - p. 352-356.

Kvasnikov E.I., Nesterenko O.A. Lactic acid bacteria and way their using. Moscow, 1976, p. 34.

Ratnikova I.A., Gavrilova N.N. Creation of probiotics for the treatment of socially significant infections / / Proceedings of the Congress "Probiotics, prebiotics, simbiotiki and functional foods", - Sankt-Peterburg, - 2007, p. 44.

Ratnikova I.A., Gavrilova N.N. Creation of complex probiotic for treatment of socially significant infections / / Proceedings of an abstracts of the International Congress probiotics. - Sankt-Peterburg, - 2009. p. 33.

Servin A.L. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens // FEMS Microbiology Reviews. - 2004. - Vol. 28, № 4, - p. 405-440.

Tulemisova K. A., Zhubanova A.A., Almagambetov K.H., Mahmudova G.S. Problem and prospects for conservation and study of microbial diversity in Kazakhstan // Biotechnology. Theory and practice. 2004. - № 4. P. 27-31.