Study of Survival During Drying of Bacterial Cells of Starter Culture for Probiotic Fermented Milk Drinks

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Abstract. Five proprietary variants of bacterial starter culture with the following species composition were studied: No. 1 – Lac. diacetilactis, Lac. cremoris, Lac. acidophilus, No. 2 – Lb. plantarum, B. adolescentis, No. 3 – Lac. cremoris, No. 4 – Lac. lactis, Lac. diacetilactis, Lb. plantarum, No. 5 – Lac. lactis, Lac. diacetilactis, Lb. cremoris. Survival was assessed immediately after freeze-drying and during storage after 30, 90 and 180 days. It was found that the lactococci included in the bacterial starter culture of variants No. 1, 3-5 after drying and during storage showed the maximum survival of bacterial cells. Their number varied within the limits: after drying – 3.3-6.5 × 10^9 CFU/g, after 180 days – 1.0-2.0 × 10^9 CFU/g. A high degree of survival was noted in L. acidophilus (variant No. 1) – the number of viable cells after drying, as well as after 180 days of storage at the level of 1.0×10^8 CFU/g. The total amount of probiotic microflora of Lb. plantarum and B. adolescentis bacterial starter culture (variant No. 2) after drying was 3.2×10^9 CFU/g, and after 180 days of storage 1.0×10^9 CFU/g. Both cultures showed high survival of bacterial cells. The number of Lb. plantarum (variant No. 4) after drying and during storage was only 1.0×10^6 CFU / g, but it should be taken into account that this culture in the starter composition is additional, while the dominant microflora is represented by lactococci.

1 Introduction

The composition of a healthy human diet includes fermented dairy products, including probiotic fermented milk drinks. Probiotics can compete with pathogens and simulate the intestinal microbiota, exhibit immunomodulatory, antidiabetic and anti-carcinogenic activity, and have other positive effects on human health [1-6]. Recent publications describe the possibility of probiotics to neutralize the effects caused by COVID-19 and reduce the risk of secondary infection [7-9].

A key role in the production of high-quality fermented milk drinks is assigned to bacterial starter cultures, including beneficial microflora. The composition of probiotic

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fermented milk drinks uses bacterial starter cultures, which are based on homofermentative and heterofermentative lactococci with the addition of lactobacilli, bifidobacteria and propionic acid bacteria [5]. It is relevant to include new strains of probiotic cultures, such as *Lactobacillus acidophilus, Lactobacillus plantarum, Bifidobacterium*, in the composition of bacterial starter cultures for fermented milk drinks.

Preservation of bacterial starter cultures is carried out by various methods – freezing, freeze drying, spray drying [10]. Freezing allows for high preservation of the properties of the starter culture, but requires expensive equipment for transportation and storage of frozen starter cultures, as well as high energy consumption for freezing. When using spray drying, the quality of starter cultures suffers, primarily the survival of bacterial cells. However, regarding spray drying, the data are contradictory, there are also opposite statements [11]. It seems that the most optimal and modern method of preserving bacterial starter cultures is still freeze drying. The use of bacterial starter cultures in dry form is justified both from an economic and technological point of view. At the same time, drying should not affect the technologically valuable properties of the starter and the survival of bacterial cells.

2 Materials and methods

Five proprietary variants of bacterial starter culture with the following species composition were studied: No. 1 – *Lac. diacetilactis, Lac. cremoris, Lac. acidophilus*, No. 2 – *Lb. plantarum, B. adolescentis*, No. 3 – *Lac. cremoris*, No. 4 – *Lac. lactis, Lac. diacetilactis, Lb. plantarum*, No. 5 – *Lac. lactis, Lac. diacetilactis, Lb. cremoris*.

For the preparation of bacterial starter cultures, sterile skimmed milk was used, into which 18-hour strains of the above cultures from the "Siberian Collection of Microorganisms" were introduced (property of the "Siberian Research Institute of Cheese Making", a division of the FSBSI Federal Altai Scientific Center for Agrobiotechnologies). The temperature of bacterial starter culture was: for variant No. 1 (32±1) °C, for variant No. 2 (37 ±1) °C, for variants No. 3-5 (30 ±1) °C. Cultivation was carried out for 16-20 hours.

Drying of bacterial starter culture samples was carried out on freeze-drying model LZ-45 (Czech Republic). The dryer is a single-chamber batch plant with a productivity of 22.5 dm^3 of moisture per 1 drying cycle.

The starter culture samples were mixed in a ratio of 7:3 with a protective medium of the composition: sucrose – 10%, sodium citric acid – 5%. After mixing with the protective medium, the resulting suspension of bacterial cultures was packaged 2 cm^3 in sterile penicillin vials. Then bacterial cultures with a protective medium (option 1-5) were frozen in a freezer at a temperature of minus (40 ± 1) °C for 12-14 hours, after which freeze-drying was carried out for 40-42 hours until starter cultures with a humidity of no more than 4% were obtained.

Dried samples of bacterial starter cultures were stored at a temperature of (6 ± 1) °C for 6 months.

Titratable acidity was determined in bacterial starter culture according to GOST 3624, gas-forming and aroma-forming activity according to the method [12].

3 Results and discussion

The results of studies on the survival of bacterial starter cells after drying and during storage after 30, 90 and 180 days are shown in Table 1.

Table 1. Survival results of bacterial starter culture microorganisms during storage for 30, 90, 180 days

Starter culture variant	Number of microorganisms, CFU g/cm^3				
	Before drying	After drying	During storage, day		
			30	90	180
1	$3,60\times10^8$	$4,43\times10^9$	$3,73\times10^9$	$2,10\times10^9$	$1,57\times10^9$
	$1,0\times10^7$	$1,0\times10^8$	$1,0\times10^8$	$1,0\times10^8$	$1,0\times10^8$
2	$5,46\times10^8$	$3,27\times10^9$	$1,72\times10^9$	$1,20\pm\times10^9$	$1,03\times10^9$
	$4,80\times10^8$	$2,83\times10^9$	$1,27\times10^9$	$7,88\times10^8$	$5,23\times10^8$
	$6,80\times10^7$	$5,23\times10^8$	$4,28\times10^8$	$4,12\times10^8$	$3,40\times10^8$
3	$1,17\times10^8$	$3,27\times10^9$	$2,93\times10^9$	$2,40\times10^9$	$1,13\times10^9$
4	$5,10\times10^8$	$5,23\times10^9$	$4,56\times10^9$	$2,77\times10^9$	$2,03\times10^9$
	$1,0\times10^5$	$1,0\times10^6$	$1,0\times10^6$	$1,0\times10^6$	$1,0\times10^6$
5	$7,87\times10^8$	$6,53\times10^9$	$5,27\times10^9$	$2,17\times10^9$	$1,30\times10^9$

Lactococci, which are part of the bacterial starter culture (variants No. 1, No. 3-5), after freeze-drying and during storage showed a high survival rate of bacterial cells. Their quantity varied within the following limits: after drying – 3.3-6.5 × 10^9 CFU/g, after 180 days of storage, their quantity decreased slightly and was at the level of 1.0-2.0 × 10^9 CFU/g.

A high degree of survival was noted in *L. acidophilus* (variant No. 1) – the number of viable cells after drying, as well as after 180 days of storage, was at the level of 1.0×10^8 CFU/g.

The total amount of probiotic microflora of *Lb. plantarum* and *B. adolescentis* bacterial starter culture (variant No. 2) after drying was 3.2×10^9 CFU/g, and after 180 days of storage 1.0×10^9 CFU/g. Both cultures showed high survival of bacterial cells.

The number of *Lb. plantarum* (variant No. 4) after drying and during the entire storage period was 1.0×10^6 CFU / g, which is the norm for this variant of the starter culture, since this probiotic culture in the general starter composition is additional, while the dominant microflora of the starter is represented by lactococci.

The technologically valuable properties of all five variants of bacterial starter culture were also studied: the time of clot formation in milk, titrated acidity, gas and aroma-forming activity. The studies were carried out before drying, after the recovery of the dried starter culture, as well as after 90 and 180 days of storage of the dry starter culture (Table 2).

Table 2. Technologically valuable properties of bacterial starter culture

No.	Period of the study of properties	Time of clot formation on milk,		Titrated starter acidity, °T	Gas-forming activity, cm	Aroma-forming activity, min
		inoculum 1 %, h	inoculum 5 %, h			
1	Before drying	16,33±0,41	4,67±0,20	97,00±2,54	5,00±0,21	5,67±0,20
	After drying	16,33±0,41	4,67±0,20	98,67±2,16	3,63±0,32	5,00±0,35
	90 days	16,33±0,41	4,67±0,20	99,67±1,78	2,80±0,25	8,17±0,21
	180 days	16,33±0,41	4,67±0,20	100,00±1,87	2,13±0,18	9,50±0,35
2	Before drying	20,00±0,35	-	93,00±2,55	-	-
	After drying	20,00±0,35	-	92,33±3,27	-	-
	90 days	20,00±0,35	-	91,67±2,48	-	-
	180 days	20,00±0,35		90,00±2,12		
	Before drying	18,83±0,41	6,50±0,35	89,33±2,86	-	-

3	After drying	18,83±0,41	6,50±0,35	88,00±1,87	-	-
	90 days	18,83±0,41	6,50±0,35	87,33±2,48	-	-
	180 days	18,83±0,41	6,50±0,35	86,67±1,78		
4	Before drying	18,50±0,71	-	118,33±2,48	3,47±0,29	5,13±0,45
	After drying	18,50±0,71	-	117,00±2,83	4,03±0,22	6,00±0,35
	90 days	18,50±0,71	-	115,67±2,94	3,07±0,11	7,83±0,41
	180 days	18,50±0,71	-	115,00±2,45	2,20±0,25	8,33±0,20
5	Before drying	18,17±0,54	6,00±0,35	95,00±1,87	4,97±0,11	4,67±0,20
	After drying	18,17±0,54	6,00±0,35	94,33±2,27	4,57±0,15	5,15±0,41
	90 days	18,17±0,54	6,00±0,35	93,33±3,19	3,50±0,28	5,33±0,20
	180 days	18,17±0,54	6,00±0,35	92,00±1,87	3,16±0,22	6,00±0,35

It was found that the dry starter (variant 1-5) after drying and during storage after recovery curdled milk at an inoculum of 1 and 5% for the same time as the liquid starter before drying. For example, liquid starter culture (variant No. 1) with 1% inoculum formed a clot on milk in 16 hours. And the same variant, when restored after drying, after 90 and 180 days of storage, also curdled milk in 16 hours. A single coagulation time of both liquid and dry starter culture after recovery was also noted with an inoculum of 5%.

On average, the titrated acidity of bacterial starter culture (variants No. 1-3 and No. 5) is 87-100 °T, and the acidity of variant No. 4, including *Lb. plantarum* and lactococci, is slightly higher – 115-118 °T.

Bacterial starter cultures (variants № 1, № 4, № 5), having heterofermentative strains of lactococci in their composition, showed high activity in gas and aroma formation after storage for 180 days.

Thus, it is proved that the dry bacterial starter culture (variants No. 1-5) in terms of the number of viable cells meets the requirements of the interstate standard GOST 34372. "Bacterial starter cultures for the production of dairy products. General specifications", and the studied variants of bacterial starter culture can be recommended for industrial use in the technology of probiotic fermented milk drinks.

4 Conclusion

It was found that the survival of bacterial cells after freeze–drying of samples of five starter cultures (No. 1 – *Lac. diacetilactis, Lac. cremoris, Lac. acidophilus*, No. 2 – *Lb. plantarum, B. adolescentis*, No. 3 - *Lac. cremoris*, No. 4 – Lac*. lactis, Lac. diacetilactis, Lb. plantarum*, No. 5 – *Lac. lactis, Lac. diacetilactis, Lb. cremoris*), as well as during storage for 6 months is quite high.

Lactococci included in the bacterial starter culture of variants No. 1, 3-5 after drying and during storage showed maximum survival of bacterial cells. Their quantity varied within the following limits: after drying – 3.3-6.5 × 10^9 CFU/g, after 180 days – 1.0-2.0 × 10^9 CFU/g.

A high degree of survival was noted in *L. acidophilus* (variant No. 1) – the number of viable cells after drying, as well as after 180 days of storage, was at the level of 1.0×10^8 CFU/g.

The total amount of probiotic microflora of *Lb. plantarum* and *B. adolescentis* bacterial starter culture (variant No. 2) after drying was 3.2×10^9 CFU/g, and after 180 days of storage 1.0×10^9 CFU/g. Both cultures showed high survival of bacterial cells.

The number of *Lb. plantarum* (variant No. 4) after drying and during storage was 1.0×10^6 CFU/g.

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