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THE STUDIES ON BIOLOGICAL ACTIVITY OF BACTERIAL PREPARATIONS DURING THEIR STORAGE UNDER DIFFERENT CONDITIONS

Summary

Application value of the preparations, employed in agriculture is connected not only with the contained therein strains of lactic acid bacteria (LAB) but also with the technological properties, determining the possibilities of utilizing the discussed preparations in a commercial form. The aim of the studies was to determine the effect of the storage conditions of the preparations of the selected strain of bacteria of the genus Lactobacillus on their biological activity during 12 months. Within the frames of the implementation of the work, the effect of the method of drying (fluidization drying or lyophilisation), the employed packaging (vacuum packing or storage in the air atmosphere) and temperature (room temperature – ca 25°C or refrigerated conditions: 10-12°C) in survivability of bacteria was determined. After 12 months of conducting the experiment, significant differences in the run of the changes in survivability were found. It refers to the tested methods as well as to the conditions of storage. Irrespectively of the employed conditions of the process, the preparations obtained with the application of fluidization drying were characterized by a lower survivability of bacteria as compared to the preparations obtained by lyophilisation. The survivability of bacteria in lyophilisates, depending on the storage temperature, varied from ca. 23% to 60% after 12 months whereas in the preparations dried by fluidization method, it amounted to 0.05-3.1%.
***Key words**: bacterial preparations, survivability of bacteria, lyophilisation, storage of bacteria.*

BADANIA AKTYWNOŚCI BIOLOGICZNEJ PREPARATÓW BAKTERYJNYCH W CZASIE PRZECHOWYWANIA W RÓŻNYCH WARUNKACH

Streszczenie

Wartość aplikacyjna preparatów stosowanych w rolnictwie związana jest nie tylko z zawartymi w nich szczepami bakterii fermentacji mlekowej, ale też z właściwościami technologicznymi determinującymi możliwości wykorzystania preparatów w formie handlowej. Celem badań było określenie wpływu warunków przechowywania preparatów wybranego szczepu bakterii z rodzaju Lactobacillus na ich aktywność biologiczną w czasie 12 miesięcy. W ramach realizacji pracy określono wpływ metody suszenia (suszenie fluidyzacyjne lub liofilizacja), zastosowanych opakowań (pakowanie próżniowe lub przechowywanie w atmosferze powietrza) oraz temperatury (temperatura pokojowa – około 25°C lub warunki chłodnicze – 10-12°C) na przeżywalność bakterii. Preparaty przechowywano przez 12 miesięcy. Po 12 miesiącach prowadzenia eksperymentu wystąpiły znaczące różnice w przebiegu zmian przeżywalności. Dotyczy to zarówno testowanych metod jak i warunków przechowywania. Niezależnie od zastosowanych warunków procesu preparaty wykonane z zastosowaniem suszenia fluidyzacyjnego charakteryzowały się niższą przeżywalnością bakterii w porównaniu z preparatami uzyskanymi poprzez liofilizację. Przeżywalność bakterii w liofilizatach, w zależności od temperatury przechowywania, wynosiła od około 23 do 60% po 12 miesiącach, natomiast w preparatach suszonych metodą fluidyzacji od 0,05 do 3,1%.
***Słowa kluczowe**: preparaty bakteryjne, przeżywalność bakterii, liofilizacja, przechowywanie bakterii*

1. Introduction

Implementation of organic (ecological) methods of farming is possible to the great degree owing to practical utilization of the results of the studies of scientific-research units, working in favour of agriculture. One of the research areas whose results are employed in agriculture includes the studies on the preparations for ensiling of roughages. The preservation of pure, non-contaminated natural environment requires elimination of acids, employed in agriculture for ensiling feeds. The application of the preparations, containing the starter cultures of lactic acid bacteria, being appropriately selected to a type of ensiled plants, is the solution which considers the requirements of ecological (organic) farms [10].

Application value of the preparation, employed in agriculture is connected not only with the contained therein strains of lactic acid bacteria but also with the technological properties, determining the possibilities of utilizing the preparations in a commercial form. Such properties include preservation of the specified biological activity of the product during the storage under different conditions which occur in practice – in the site of the direct customer as well as of the distributors of a given product. The results of the studies as being presented in this paper are dedicated to the mentioned problem.

In the Institute of Agricultural and Food Biotechnology, there are produced the bio-preparations, used in agriculture for preservation of feeds and those ones employed as animal probiotics and also, as the preparations for preservation of raw materials for biogas-manufacturing plants and preparations for ensiling vegetables and initiating fermentation of bakery starters. The discussed preparations have been the result of the research work carried on for many years. Until 2015, they had a form of granulates and were obtained by the fluidization drying method. The stability (shelf-life) of the discussed preparations, especially during their storage at a room temperature, was not satisfactory – the survival of bacteria after 12 months of storage was found on the level below 1%.

In order to improve the survivability of bacteria in biopreparations during their storage, the studies on preservation of biomass by a sublimation drying method were undertaken. Lyophilisation is characterised by many advantages which make it almost ideal method for preservation and preparation to a long storage of industrial strains [5]. Bacterial strains, as preserved by the discussed method, keep the biochemical, morphological and immunological properties of initial cells even after a long-time storage period [3]. The impact of particular physical factors on microbial survivability is not univocal. The scientists are consistent only in the opinion that the additive of protective substances improves the survivability of microorganisms [2, 4, 6]. It is recognised that the protective substances counteract the cell-inactivating mechanisms and namely: increase of electrolytes' concentration and ice crystallization in their interior [7]. The exemplified scientific reports indicate e.g. that the survivability of microorganisms during the storage may be affected even by dry matter of biomass, subject to lyophilisation [1]. Porter et al. found considerable differences in survival of bacteria during their storage, depending on temperature (4 or 25°C) and atmosphere of environment (vacuum, air of nitrogen atmosphere) [9].

The purpose of the present work was to determine the effect of storage conditions of the dried by fluidization method and lyophilised biomass of *Lactobacillus plantarum* KKP 593 bacteria on the number of surviving bacteria.

2. Methodology

The studied organism is *Lactobacillus plantarum* K KKP/593/p – the microbial strain, obtained by the selection method at the Laboratory of Fermentation Technology of the Institute of Agricultural and Food Biotechnology, being characterized by the capability of synthesizing the extracellular amylolytic enzymes and dynamic synthesis of lactic acid. It is employed as basic biological component of the preparations, stimulating the process of ensiling the feeds, especially those containing starch carbohydrates. The preparations, obtained with the application of the mentioned strain enable production of feed silages from such plants as grasses, alfalfa (lucerne), clover, maize (grain and whole plants), other cereals, raw potatoes, and from by-products of agri-food industry such as beet pulp and distillery decoction. The strain *Lactobacillus plantarum* K KKP /593/p is also characterized by probiotic properties. As a result of the studies concerning the antibacterial properties of the discussed strain (micro-aerobic conditions) it was found that it revealed the capacities of inhibiting the development of pathogens, isolated from alimentary tract of sick animals.

2.1. Obtaining material for tests

The multiplied bacterial biomass was separated from post-cultivation liquid by centrifugation; then it was divided into two parts: the first one was dried by fluidization method (the comparative material was obtained by the method employed at the Fermentation Technology Department of IAFB for production of biopreparations) and the other one was subjected to dehydration by the sublimation method.

Dehydration performed by fluidization technique was as follows – the experimental preparations were obtained while spraying the bacterial biomass onto the granulated carried during drying process in fluidized bed, at temperature not exceeding 35°C. The parameters of drying were: air relative humidity 30–35°C (air is dried using drying device), temperature of the air inside the fluidal bed – the changes increasing from initial value of 26-28°C until the final one 33-34°C. Final humidity of the preparations after drying is from 4.4 to 5.5%, time of drying is 30-35 min.

Dehydration by the sublimation method was conducted in semi-technical freeze-dryer; working temperature in crystallizer's chamber: -55°C, time of the process ca. 48 h. Final humidity of the preparation was 2-3%.

The dried preparations were vacuum-packed in barrier PA/PE film bags or in small polyethylene jars supplied with moisture absorbers (storage in the air atmosphere). The samples from all variants were in parallel stored under refrigerated conditions (10-12°) or at a room temperature (ca 25°C) for 12 months. The application of the specified packages was connected with the testing of different storage conditions, as for the storage under vacuum, it is necessary to apply the completely hermetic packages, such as barrier bags.

The employed microbiological methods: determination of lactic acid bacteria (LAB) by plate method on MRS medium acc. to standard PN –EN 15787:2009 [8].

The employed analytical methods: determination of dry matter content – by thermal-gravimetric method, using weighing-drying device by Sartoris company.

2.2. The results of the studies

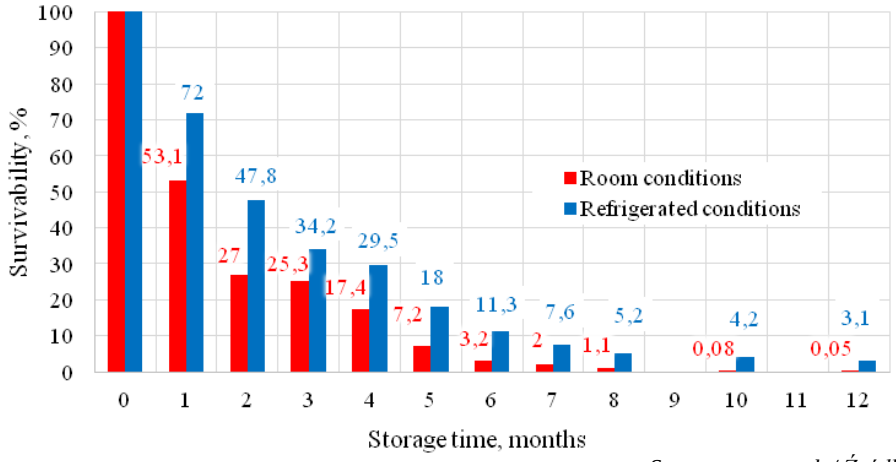
Initially, the samples from each experimental variant were collected in one month intervals and in the period of 8–12 months – each two months. The initial bacterial count in the preparations subject to fluidal drying was equal to 6.0 x 10^{10} CFU/g whereas in the preparations dried by sublimation method, it amounted to 1.79 x 10^{11} CFU/g. Due to different values of the initial bacterial counts in the preparations, the direct comparison of the results of inoculation, obtained systematically during the duration of the storage experiments would be purposeless, therefore, the characterization of the occurring changes was carried out using the survivability, expressed in percentage, counted as ratio of actually determined bacterial count and their initial value in the preparation (directly after drying) and multiplied by 100. The results of each experimental variant were shown on separate diagrams, marked with the numbers from 1 to 4.

Fig. 1 refers to fluidization drying and the storage of the preparations in the air in the polyethylene containers. The mentioned results constitute the comparative material because the preparations, existing before commencement of the tests were obtained by the discussed method and stored under the discussed conditions. In such case, there was found a quite quick loss of biological activity (expressed as number of surviving bacteria in CFU/g) of the preparations during the first 6 months of the storage to the level of 11.3%, that is, by one order of size (from 10^{10} CFU/g to 10^{9} CFU/g) in the case of storage under the refrigerated conditions and to the level of 3.2% of the initial bacterial count in the case of storage at the room temperature, i.e. by two orders of size. Respectively, after 12 months of the storage in refrigerated conditions, the survivability amounted to 3.1%, i.e. was lowered by 2 size orders and in the case of the storage at the room temperature, it was lowered to 0.05%, i.e. by 3 orders of size.

The diagram found on Fig. 2 illustrates the changes in biological activity, occurring in the same preparation, being stored in vacuum (plastic film bags). The application of vacuum packages had a small effect on the results. The changes in the activity had the run similar to that one presented in Fig. 1, especially up to the third month of the storage; after the mentioned period, more distinct differences appeared, unfavourable for the storage under vacuum conditions. In the case of the storage of the preparations in refrigerated temperature for 12 months, the survivability was equal to 0.07%, that is, the measured parameter was lowered by 3 orders of the size; in the case of the storage at the room temperature, the survivability was 0.005%, i.e. the decline in the bacterial count was equal to 4 orders of the size.

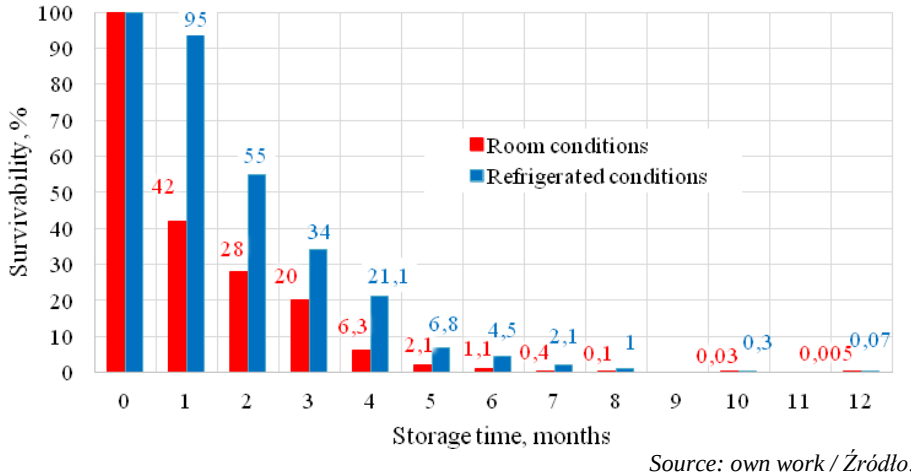
The application of drying by the sublimation method allowed obtaining considerably better results. The survivability of lyophilised bacteria, being then stored in the polyethylene containers (Fig. 3) was lowering considerably slower than during the earlier described experiments, concerning fluidized bed drying. After 12 months of the storage under the refrigerated conditions, it was equal to 58.9%, that is, a decline in activity, being expressed as survivability, had place only by one order of the size. Also, in the case of the storage of lyophilisates at the room temperature, the obtained results were considerably better as compared to the experimental data, relating to the preparations, dried by the fluidization technique - the survival of bacteria amounted to 23.2% what should be recognized as a very good result. The change of the storage conditions into vacuum storage has not brought any significant changes in survivability of bacteria, irrespectively of the employed environment temperature. After 12 months of the storage in the refrigerated conditions, the survivability was equal to 48.5%, i.e. it was lower by 10% as compared to the storage in the air atmosphere whereas at the room temperature, the survivability amounted to 22.5%, i.e. it was almost identical as that one obtained in the case of the storage in the air atmosphere for 12 months.

The results of the studies, obtained in the present work indicate that the developed method of dehydration gives equally good results in the case of the storage in vacuum conditions and in the air atmosphere what may be recognized as the advantage of the method; it gives greater possibilities for packing of the preparations obtained by the discussed method, so it rises also their commercial attractiveness. On the other hand, the relationship between the survivability and the temperature of the storage has been confirmed; better results of bacterial survivability in the preparations stored in refrigerating conditions as compared to the room temperature were obtained in all experimental variants of the present work.



Source: own work / Źródło: opracowanie własne

Fig. 1. Survivability of lyophilised *Lactobacillus plantarum* K KKP 593 bacteria, dried by fluidization method, during their storage in small bottles
Rys. 1. Przeżywalność suszonych fluidyzacyjnie bakterii Lactobacillus plantarum K KKP 593 w czasie przechowywania w buteleczkach



Source: own work / Źródło: opracowanie własne

Fig. 2. Survivability of lyophilised *Lactobacillus plantarum* K KKP 593 bacteria, dried by fluidization method during their storage in sachets
Rys. 2. Przeżywalność suszonych fluidyzacyjnie bakterii Lactobacillus plantarum K KKP 593 w czasie przechowywania w saszetkach

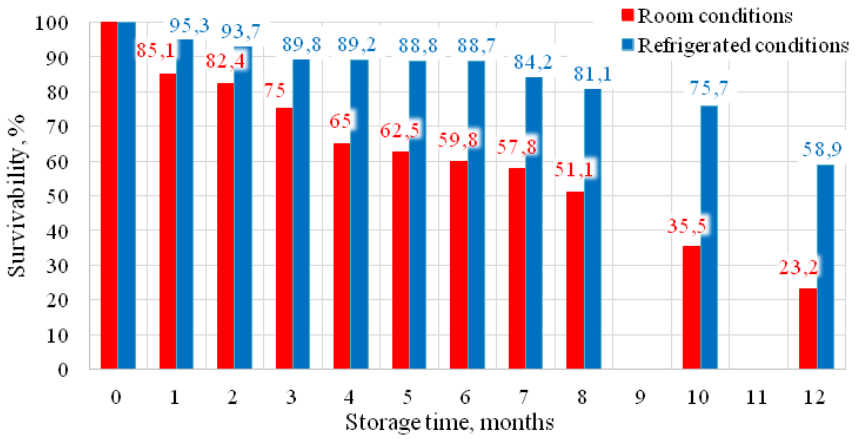


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Fig. 3. Survivability of lyophilised *Lactobacillus plantarum* K KKP 593 bacteria during their storage in small bottles
Rys. 3. Przeżywalność liofilizowanych bakterii Lactobacillus plantarum K KKP 593 w czasie przechowywania w buteleczkach

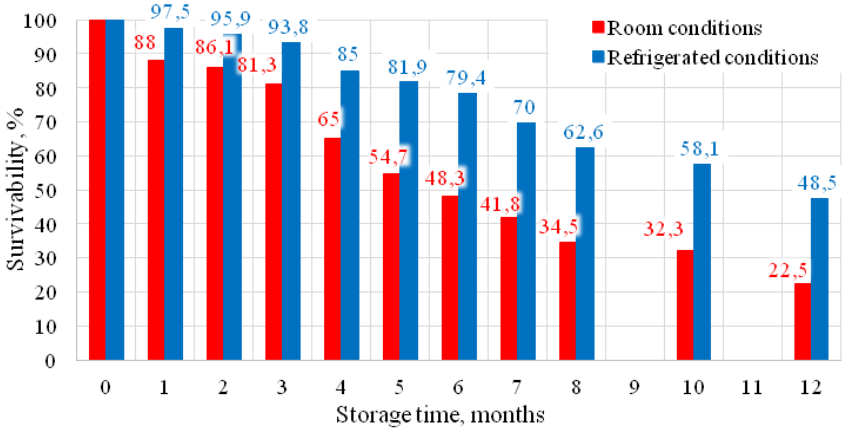

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Fig. 4. Survivability of lyophilised *Lactobacillus plantarum* K KKP 593 bacteria during their storage in sachets
Rys. 4. Przeżywalność liofilizowanych bakterii Lactobacillus plantarum K KKP 593 w czasie przechowywania w saszetkach

3. Conclusions

1. The developed method for dehydration of the examined bacterial strains, as employed in biopreparations for ensiling feeds and in probiotic preparations enables obtaining the products with a shelf-life (stability) acceptable by distributing companies; after 12 months of the storage under the refrigerated conditions, the survivability of bacteria was equal to ca. 59.0%.
2. Irrespectively of the experimental variant, the preparations produced with the application of fluidized drying were characterized by considerably worse survivability of bacteria as compared to the preparations obtained by lyophilisation method.
3. The developed hydration method has been introduced in semi-technical line for manufacture of biopreparations of Fermentation Technology Department of IAFB what increases the bacterial survival of drying process; it allows lowering the price of the sold products and rises their market attractiveness due to the improvement of shelf-life during their storage for 12 months.

4. References

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