Studies on the growth of micrococci and lactobacilli with reference to their survivability upon freeze-drying

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The growth conditions were studied and the age was determined of bacterial cultures of micrococcus strain M_{95}, and lactobacillus strain L_4, at which age the best survivability could be attained upon freeze-drying.
It was found that bacterial cultures in the end of the exponential phase and in the initial stationary phase of growth, at a temperature of 26°C, are at the most suitable age for freeze-drying. The bacterial cell number in the end of the exponential growth phase reaches 10^9 cells/ml for the micrococcus, and 10^{11} cells/ml for the lactobacillus. Upon freeze-drying, 100% survivability was achieved in a suitable protecting medium, and that is important in the preparation of freeze-dried preparations for the manufacture of ripening meat products.

Untersuchungen über die Entwicklung von Mikrokokken und Laktobazillen im Zusammenhang mit ihrer Überlebungsfähigkeit bei der Gefriertrocknung

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Es wurden die Entwicklungsbedingungen untersucht, sowie das Alter der Bakterienkulturen von Mikrokokkus Stamm M_{95} und Laktobazillus Stamm L_4, bei dem die beste Überlebungsfähigkeit nach der Gefriertrocknung erreicht wird, bestimmt. Es wurde festgestellt, dass das geeigneteste für eine Gefriertrocknung Alter der Bakterienkulturen am Ende der exponentiellen und in der primären stationären Entwicklungsphase bei einer Temperatur von 26°C ist. Die Bakterienzellen erreichen am Ende der exponentiellen Entwicklungsphase eine Anzahl von 10^9 Zellen/ml beim Mikrokokkus und 10^{11} Zellen/ml beim Laktobazillus. Nach Gefriertrocknung wurde eine 100 %-ige Überlebungsfähigkeit in einem geeigneten und begünstigenden Medium erreicht; dies ist bei der Zubereitung von gefriergetrockneten Präparaten zur Herstellung von reifenden Fleischprodukten von Bedeutung.

7.12

Etude sur le développement des microcoques et des lactobacilles du point de vue de leur survie lors de la lyophilisation

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On a étudié les conditions de développement des cultures bactériennes d'une microcoque souche M_{95} et d'un lactobacille souche L_4 et on a déterminé l'âge auquel elles présentaient une meilleure survie après la lyophilisation.
On a constaté que les cultures bactériennes avaient l'âge le plus favorable à la lyophilisation à la fin de la phase exponentielle et dans la phase primaire stationnaire à une température de 26°C. A la fin de la phase exponentielle de développement le nombre de cellules bactériennes était de 10^9 cel./ml pour la microcoque et de 10^{11} cel./ml pour le lactobacille. Après la lyophilisation on observait 100 % de survie dans un milieu convenable protecteur ce qui était important pour l'élaboration de préparations lyophilisées, destinées à la fabrication de produits carnés maturants.

Исследования развития микрококков и лактобацилл в связи с их живучестью при лиофилизации

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Изучены условия роста и определен возраст бактерийных культур микрококка штамма $М_{95}$ и лактобациллы штамма $Л_4$, в котором можно достичь лучшей живучести после лиофилизации.
Установлено, что бактерийные культуры в конце экспоненциальной и в первичной стационарной фазе роста при температуре 26°С находятся в наиболее подходящем возрасте для лиофилизации. Количество бактерийных клеток в конце экспоненциальной фазы роста достигает 10^9 клеток/мл для микрококка и 10^{11} клеток/мл для лактобациллы. После лиофилизации достигнута 100% живучесть в подходящей защитной среде, что является важным при приготовлении лиофилизированных препаратов для производства созревающих мясопродуктов.

7.12

Studies on the growth of micrococci and lactobacilli with reference to their survivability upon freeze-drying

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The age of bacterial cultures is one of the factors affecting their survivability upon freeze-drying.

Blankov and Klebanov (2), Arkad'eva et al. (1), Naylor et al. (11) and others have found, that the most favourable age of the microorganisms for freeze-drying is the end of the exponential phase and the initial stationary phase of their development. Takano (12) and others report that young bacterial cells are much more susceptible than older ones to the effects of sublimation drying. Morichi (10) indicates a higher survivability of lactic acid streptococci upon freeze-drying in the end of the exponential growth phase. A number of other authors (3, 5, 6, 7, 8, 9, etc.) are also of the opinion that the most suitable time for freeze-drying lactic acid bacteria is the end of the exponential phase and the beginning of the stationary one.

The objective of the present work was to establish the growth phases of micrococci and lactobacilli applied as pure starter cultures in the production of fermenting meat products, with reference to their survivability upon freeze-drying.

Materials and Methods

The studies were carried out with Lactobacillus, L_4, and Micrococcus, M_{95}, isolated from raw-dried sausages and classified as L. plantarum and Micrococcus varians.

Cultivation was effected in nutrient media suitable for the two species, at temperatures of 26° and 15°C., which are close to production conditions of ripening and drying for fast ripening (fermented), and raw-dried sausages.

For inoculation, 24-hour cultures were used, with a concentration of $\bar{x} = 4{,}5 \cdot 10^9$ cells/ml, in quantities of 0,1 and 0,01 % related to the nutrient media, which were previously tempered at the experimental temperature. The number of bacterial cells was determined by power dilutions, at 1 hour intervals, with correction of numerical values according to McKredy. The determinations were made in 3 to 5 replicas (n = 3 to 5). Growth phases were determined according to Tarkov (4).

Nonfat dry milk was added to the cultures to be freeze-dried, as a protecting medium. The survivability of bacterial cells was established before and after the freeze-drying of cultures in the end of the exponential and in the early stationary phase of their growth. Sublimation drying was performed at -30° ÷ -35°C., and subsequent drying, at 25 ÷ 35°C.

Results

In Figure 1, the curves are plotted, that show the growth phases of Lactobacillus L_4 at cultivation temperatures of 15 and 26°C., with the introduction of different amounts of the inoculum. It is obvious that the different amounts of culture introduced affect the duration of the lag phase, but do not affect the subsequent growth phases. At a cultivation temperature of 26°C and an inoculation level of 0,01% of the broth culture, the lag phase (I) lasts 4 hours, and at the 0,1% level, 3 hours. The exponential growth phase (IV) lasts to the 10th

hour. On the 8th hour, the amounts of bacterial cells become equal in the two experiments with different percentages of the broth cultures introduced. In individual cases, a certain variation is observed up to the 28th hour in the phase of slowed-down acceleration (V), when the stationary phase (VI) begins. In all experiments, a decrease in lactobacilli numbers was shown between the 36th and the 42nd - 44th hour, by more than one power, and a second exponential phase (IV) was found towards the 48th hour. That could be explained by the accumulation of metabolite products and an acidification of the medium. At a given moment, however, there starts again an intense reproduction of the lactobacilli, that adapted themselves to the new conditions, and, after reaching M concentration (the maximum possible concentration), there begins the exponential dying-off till the 54th hour, after which a clearly manifested phase of slowed-down dying-off (VII) is observed, till the 80th hour, and a phase of exponential dying-off (VIII) - after the 80th hour.

Interest is aroused by the early stationary phase (VI), in which combinations of 4 different growth phases are observed.

From the studies of the growth characteristics of Lactobacillus L_4, and the results obtained, it was considered right to hand over the cultures for freeze-drying on the 48th hour, when there is a maximum number of cells in the active mature form, ensuring a higher resistance under the conditions of freezing and sublimation drying.

At a cultivation temperature of 15°C., the duration of the lag phase with the inoculation of 0,01 % of the broth culture, is 19 hours, and with 0,1%, 15 hours; the exponential phase (IV) is of a duration of 21 and 25 hours, respectively: a short initial stationary phase (VI) of 8 hours, a phase of slow acceleration (V) - 16 hours, an initial stationary phase (VI) - 14 hours, and a phase of exponential dying-off (VIII), which sets in after the 78th hour from the inoculation. At that cultivation temperature, the amount of the lactobacilli was found to approach, after the 40th hour, that of the ones cultivated at 26°C., and after the 54th hour it is greater. That could be explained by the lower accumulation of metabolites in the medium at lower cultivation temperatures, which gives possibilities for a weaker inhibition of the reproduction process.

Figure 2 constitutes a graphic representation of the growth phases of Micrococcus M_{95}. Upon cultivation at 26°C., 4 growth phases are clearly manifested: a lag phase (I) of 6 hours, with the introduction of 0,01 %, and of 4 hours with 0,1% of broth culture, an exponential phase (IV) - till the 12th hour, and a prolonged early stationary phase (VI) - till the 72nd hour, after which the phase of slowed down dying-off begins (VII).

The application of a 48-hour culture was also established for Micrococcus M_{95} with a view to freeze-drying: then, the cells are in a period of maturity and greater resistance. According to Tarkov (4), the resistance of bacterial cells at that age is higher.

Upon 15°C. cultivation, a longer lag phase was also found: 22 hours for 0,01% of broth culture introduced, and 19 hours for 0,1%. The results obtained indicate that micrococci adapt themselves more slowly at lower temperatures, than the lactobacilli. The exponential phase (IV) lasts till the 32nd hour, after which the number of bacterial cells is equalized with the number of those at the same age, cultivated at 26°C. From the 32nd to the 72nd hour, a phase of slowed down dying-off (VII) is observed, without any initial stationary phase (VI) to be established.

From the experiments carried out repeatedly, a 100% survivability upon freeze-drying was found for the cultures of Lactobacillus L_4 and Micrococcus M_{95} in the end of the exponential and the early stationary phase of their development.

Conclusions

1. It was found that a suitable freeze-drying age of the bacterial cells of Micrococcus M_{95}

and Lactobacillus L_4 is the end of the exponential and the early stationary phase.

2. The number of bacterial cells in the end of the exponential growth phase reaches 10^9 cells/ml for the micrococcus and 10^{11} cells/ml for the lactobacillus.
3. On freeze-drying the cultures in a protecting medium, nonfat dry milk, a 100% survivability of bacterial cells is achieved.

References

1. Arkad'eva, Z.A., E. Kozlova, T. Kovalenko. Prikl. biokhimiya i mikrobiol., 11, 4, 519,1975
2. Blankov, B.I., D. Klebanov. Primenenie liofilizatsii v mikrobiologii, Mezgiz, Moskva,1961
3. Lukovnikova, L., I. Osipova. Molochnaya promyshlennost', 7, 17-19, 1967
4. Tarkov, M. Mikrobiologicheskie metody otsenki iskusstvennykh pitat. sred. Izd.SHTIINTSA, Kishinev, 1972
5. Accolas, J., J. Auclair. J. of Dairy Technol., 23, 1, 22-29, 1968
6. Accolas, J., J. Auclair. La Rev.gen.de bacterie, 63, 1, 21-25, 1972
7. Cowman, R., M. Speck. J.Dairy Sci., 48, 1531-1532, 1965
8. Cowman, R., M. Speck. Cryobiology, 5, 5, 291-299, 1969
9. Maxa, V., M. Teply. Prumysl Potravin, 9, 465, 1960
10. Morichi, T., FIL. Scientific conference in Tokyo, Japan, 56 An.Session, 1-17, 1972
11. Naylor, H., P. Smith. J. Bacteriol., 52, 5, 565-573, 1946
12. Takano, M., J. Sado, T. Ogawa, H. Terui. Cryobiology, 10, 5, 440-445, 1973

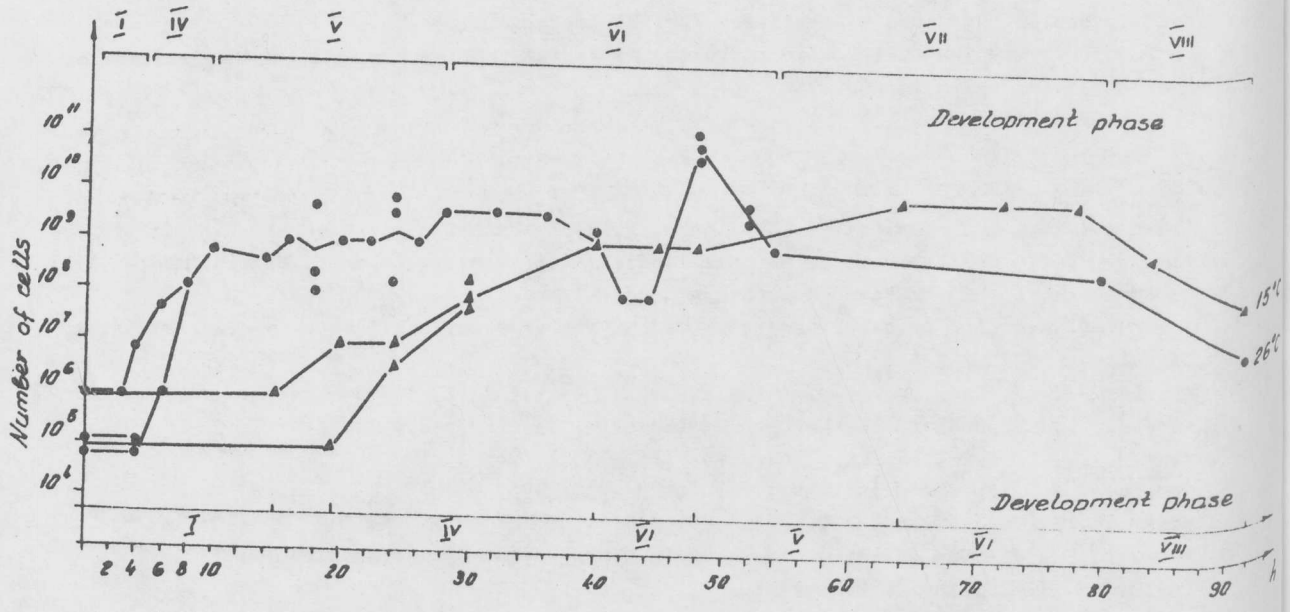

Fig. 1. Development phase of lactobacilus, strain L_4

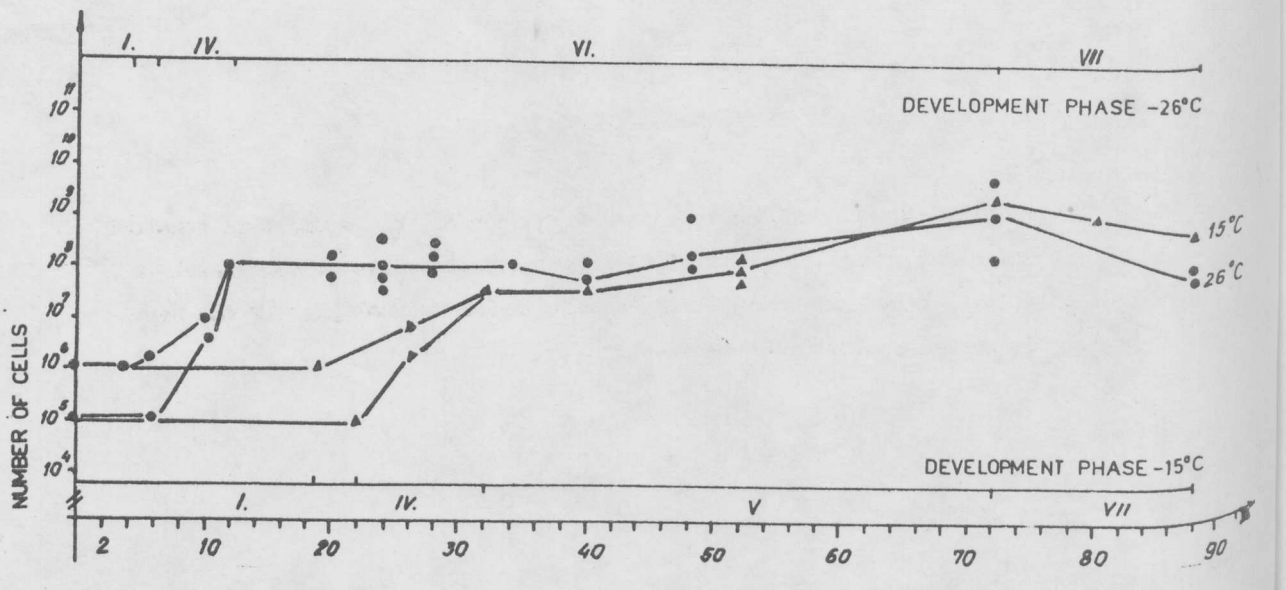

FIG. 2 DEVELOPMENT OF MICROCOCUS, STRAIN M_{95}