

INFLUENCE OF THE CULTIVATION CONDITIONS ON YEAST STRAINS SURVIVAL AFTER LYOPHILIZATION

Todor Donev^{1*}, Irena Savova¹ and Anna Kujumdzieva²

¹National Bank for Industrial Microorganisms and Cell Cultures, 1113 Sofia, P.O.Box 239, Bulgaria; ²The Sofia University, Biological Faculty, Department of General and Industrial Microbiology, 8 "Dragan Tsankov" st., 1421 Sofia, Bulgaria

Summary

*The growth curves of strains belonging to genera *Candida* and *Saccharomyces* grown through surfaced and submerged cultivation and their survival after lyophilization have been studied. Conclusions of practical importance have been drawn. It was found out that three-day-old cultures cultivated in solid medium possessed maximal resistance to freeze-drying. Nevertheless the cultures obtained by submerged cultivation were with considerably lower survival the best results were obtained at 12 h and 18 h of their development.*

Introduction

The importance of yeast for the industry outlines a special interest towards the methods for their preservation. Freeze-drying is one of the most convenient for industrial purposes [1,2,3,12,13]. To perform a satisfying result lyophilization it is necessary to carry out profound investigations of the yeast cultures, because of their sensibility to this kind of treatment.

The microorganisms viability during conservation and storage is influenced by their nature, cultivation conditions, age, culture and protecting medium concentrations, regime of

the process, etc. [7,8,10].

In order to preserve the strain specific characteristics it is necessary to apply routine approaches, based on preliminary tested models. The main task is maximum number of initial cells to be kept and the first tool available is cultivation.

The aim of the present work was to follow the growth dynamics of strains belonging to genera *Candida* and *Saccharomyces* grown through surfaced and submerged cultivation and their survival after lyophilization in order to make conclusions of practical importance.

Materials and Methods

Microbial cultures, media and protectants. Strains from genus *Candida* - *C. lipolytica* 1-7 (NBIMCC 687), *C. boidinii* 77-1 and genus *Saccharomyces* - *S. cerevisiae* 53 (NBIMCC 180), *S. cerevisiae* K₁₂ were used. The strains were grown on Rider medium with the following composition g/l: glucose - 20, (NH₄)₂SO₄ - 3.0 MgSO₄.7H₂O - 0.7, NaCl - 0.5, CaCl₂ - 0.4, KH₂PO₄ - 1.0, K₂HPO₄ - 0.1, yeast extract - 0.1, MnSO₄.5H₂O - 0.01, ZnSO₄.7H₂O - 0.07, CuSO₄.5H₂O - 0.01,

FeSO₄.7H₂O - 0.05, pH adjusted to 6.5 and on solid medium - malt extract, pH 5.6. The cell concentration for lyophilization was 2-5 x 10⁷ cells/ml. The cryoprotecting medium used was sucrose - 10% and gelatine - 1.5%. The lyophilizates were rehydrated with 0.9% water solution of NaCl at 18-20°C.

Cultivation conditions. The growth temperatures were 26-28°C for the members of genus *Saccharomyces* and 28-30°C for genus *Candida*. In the first group of experi-

ments the strains were grown through submerged cultivation on "Gallencamp" rotary-shaker at 220 rpm in Erlenmayer flasks (500 ml) with 100 ml working volume. The development of the culture was followed until the 42 h. Samples for analysis had been taken every 3 h. In the second group of experiments the strains had been grown through surfaced cultivation on solid medium for 160 h and samples for analysis had been taken every 8 h.

Methods. The freeze-drying was performed according to a standard method [12,13] on "SMH.15-Usifroid" apparatus with

0.2 ml suspension in ampoules which were closed under vacuum after lyophilization.

The viability of the cells was estimated indirectly by counting of colonies on solid medium in Petri dishes, 48 h after inoculation of logarithmically diluted cell suspension.

The residual concentrations of glucose (C) [11], inorganic phosphorous (P) and nitrogen (N) [6], as well as pH and the dry weight (DW) of the samples were determined during the submerged cultivation. The residual moisture in the lyophilizates was measured gravimetrically after further drying at 105°C to constant weight.

Results and Discussion

The influence of the submerged cultivation on the viability after lyophilization of strains belonging to genera *Candida* and *Saccharomyces* was tested in the first group of experiments. The growth curves of the strains, as well as their viability after freeze-drying, are presented in fig 1. In these cultivation conditions the strains of genus *Candida* showed relatively better viability after lyophilization of the log-phase cells. In the 12 h old cultures *C. boidinii* 77-1 (a) expressed 19.46% viability while *C. lipolytica* 1-7 (b) - 12.50%. The same was the tendency in the 18 h old culture of strains of genus *Saccharomyces* but the viability percentage was considerably lower: *S. cerevisiae* 53 (c) - 1.63% and *S. cerevisiae* K₁₂ (d) - 0.28%. A sharp decrease of this parameter was observed during the next hours. During this period the microorganisms entered the stationary phase of their development and it probably effected the cell resistance to lyophilization.

The analysis of the residual N, P and C concentrations in the medium during submerged cultivation showed that after consumption of the glucose there was enough quantity of N and P in the medium. This fact indicated that the ceasing of the cell growth was due to glucose deficiency which could lead to the lack of some important components (i.e. trehalose) in the starving cells during the stationary phase and to the decrease in the cell resistance to lyophilization [2,5,9].

The higher demonstrated viability of the cells during logarithmic growth phase of the tested microorganisms can be connected with reactivation from lyophilized condition

and the contact of the cell with oxygen [6]. In this context it sounds reasonable, that the treated cells in this growth phase are more resistant, because of the presence of intracellular active enzyme defence mechanism.

The development of the strains on solid medium and their viability after lyophilization were tested in the second group of experiments. The results for the strains viability before and after the drying are presented in fig 2. The registered maximal viability was considerably higher in comparison with that of the strains cultivated in submerged way. *C. boidinii* 77-1 (a) kept 36.74% of its viability on the 80 h and *S. cerevisiae* K₁₂ (b) - 8.72% on the 64 h. The viability slightly decreased during the next hours and at the end of the experiment (160 h) the viability values were 30.11% for *C. boidinii* 77-1 and 5.38% for *S. cerevisiae* K₁₂.

On the basis of the obtained results it could be speculated that the difference in the resistance of surface and submerged cultures to lyophilization, is due to a difference in their physiological status.

Thus, in the lyophilization of yeasts of genera *Candida* and *Saccharomyces* for collection purposes three-day-old cultures obtained through surfaced cultivation on solid medium have to be preferred. This fact has been proved many times in the routine work of the National Bank for Industrial Microorganisms and Cell Cultures. When conservation of big volumes of starter cultures for industrial purposes is necessary a submerged cultivation have to be used, because the low viability can be compensated with a greater mass of lyophilizate.

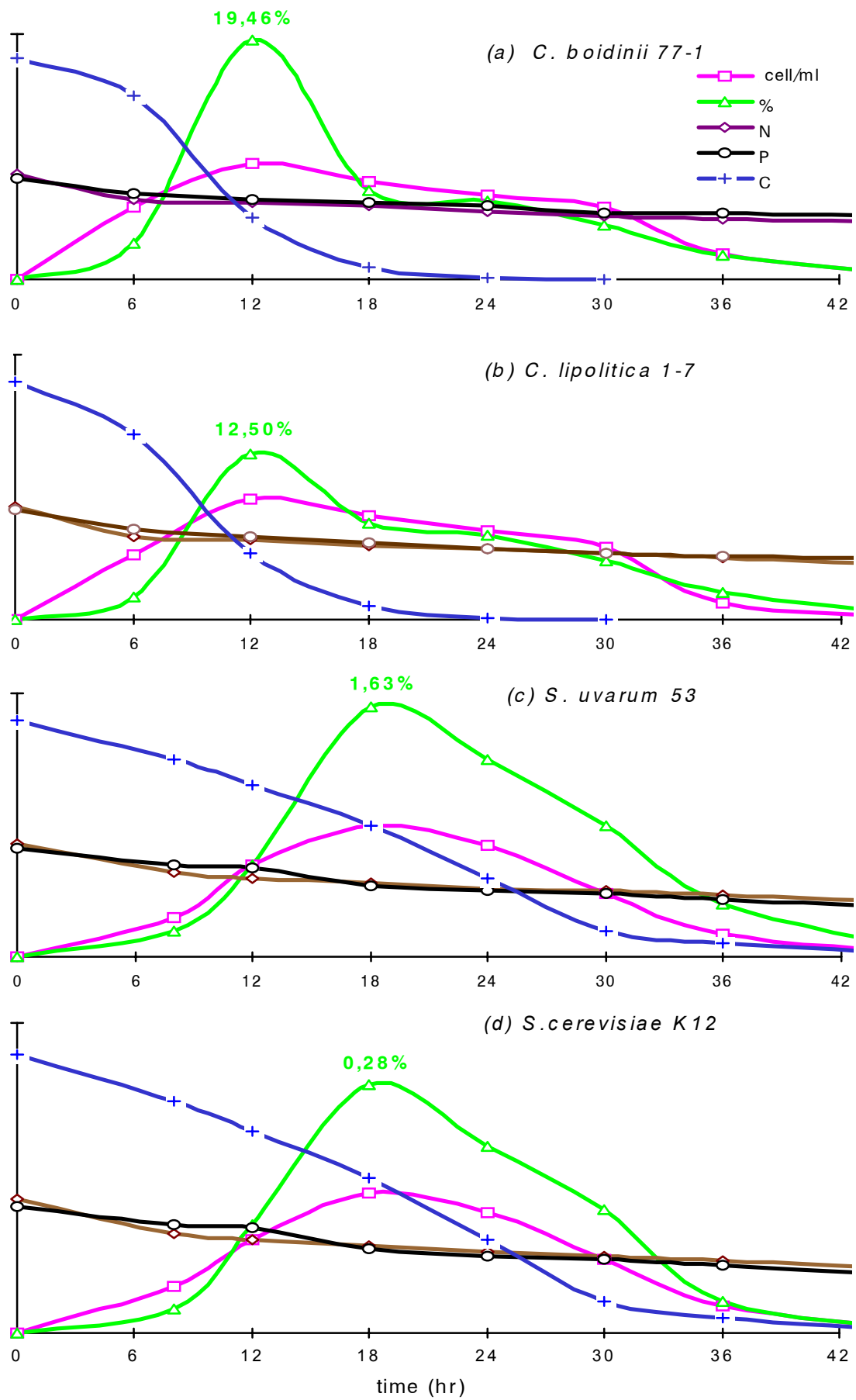


Fig. 1. Growth curves and influence of submerged cultivation (N, P and C availability) on survival (%) of genera *Candida* and *Saccharomyces* cultures after lyophilization.

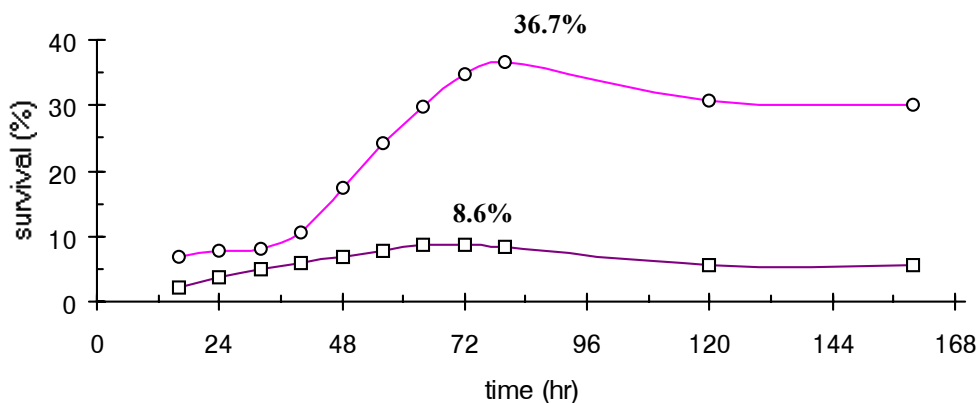


Fig. 2. Survival of lyophilized at different age (h) cultures obtained on solid medium: *S. boidinii* 77-1 (—○—) and *S. cerevisiae* K12 (—□—).

References

1. Becker, Ì. À., B. E. Damberg, À. I. Rapoport, 1981. *Anabiosys of microorganisms*, Riga: Zinatne, 203-213 (in Russian).
2. Becker, Ì. À., À. I. Rapoport, L. V. Kalakut-skii, 1986. *Cessation of the cells' vital activity*, Riga: Zinatne, 100-119 (in Russian).
3. Damberg, B. E., À. À. Upit, 1973. *Anabiosys and preanabiosys of microorganisms*, Riga: Zinatne (in Russian).
4. Damberg, B.E., I.L. Kralliss, M.J. Beker, 1983. *The Effect of Dehydration on Some Energetic Systems of Yeast Saccharomyces Cerevisiae L-1*. 3rd Symposium. of Socialist Countries on Biotechnology, Bratislava, CSSR, abstracts, B5, 4.
5. Grba, S., E. Oura, H. Suomalainen, 1979. *Finn. Chem. Lett.*, **2**, 61-64.
6. Herbert, P., P.J., Phipps, R.E., Strange, 1971. *Meth. Microbiol.*, **5B**, 114-137.
7. Kirsop, B.E., 1974. *J. Inst. Brew.*, **80**, 565-570.
8. Kirsop, B.E., 1985. *II National School of Cryobiology and Freeze-drying*, 1-10 VIII, Tolbouchin, Bulgaria.
9. Lillie S.H., J.R. Pringle. 1980. *J. bacteriol.*, **143**, (3), 1384-1394.
10. Savova, I., T. Donev, 1987. *VIII Congres of the Microbiologists in Bulgaria*, 21-23 X, Varna, Bulgaria.
11. Somogyi, M.J., 1952. *J. Biol. Chem.*, **195**, 19-32.
12. Tepavicharova, I., Ò. Donev, 1987. *Methods for preservation of microorganisms*, Sofia: NBIMCC (in Bulgarian).
13. Tsvetkov, Ts., 1981. Sublimate consrevation of biological materials, *In: Actual problems of cryobiology*, N. S. Pushkar and À. Ì. Belous (eds.), Kiev: Naukova Dumka, 428-482 (in Russian).