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INFLUENCE OF THE FREEZING RATE ON THE SURVIVAL OF STRAINS *SACCHAROMYCES CEREVISIAE* AFTER CRYOGENIC PRESERVATION

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Summary

Experiments for determination of the influence of the freezing rate on the viability preservation of brewery yeasts Saccharomyces cerevisiae were made. Six freezing rates were studied (0.3, 0.6, 1.0, 10, 30 and 300^{o}C x min^{-1} ranging from 20^{o}C to minus 196^{o}C) and subsequent thawing at three different temperature values – 20, 27 and 37^{o}C was performed. It was established that the investigated strains 179, 181, 184 had maximum preserved their viability by freezing rate of 0.6^{o}Cxmin^{-1} and subsequent thawing in water bath at 37^{o}C. These results are applied for cryogenic preservation of brewery yeasts in the National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC).

Introduction

The freezing rate unconditionally has influence on the survival of the treated yeast suspensions [7÷13]. The lack of particular, confirming data concerning the relation between the freezing and thawing rates and the preservation of brewery yeasts of the species *Saccharomyces cerevisiae*, as well as our own investigations have provoked the necessity of this study [2, 6, 11]. For this purpose a culture, obtained by a combined method as previously described [5] and protected with 10 % glycerol (v/v), was used.

Materials and methods

Microorganisms. Industrial strains from the collection of the National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC) with numbers 179, 181, 184, used for brewery production were studied.

Nutrition medium. GPYA - glucose10 g, peptone 5.0 g, yeast extract 5.0 g, agar 20 g, distilled water 1 l, pH 6.

Protective medium. Glycerol 10 % (v/v).

Package. Cryotubes with volume of 2 cm^{3},

in which 1 ml cell suspension is distributed.

Cultivation. The investigated strains were grown at temperature 27^{o}C in glass tubes with GPYA medium. The concentration of the inoculum was 10^{8}cells/ml [5]. The well grown for 72 hours on slope agar culture was poured off with glycerol (10 %) as protective medium.

Freezing. Experiments with the protected cell suspension were carried out, by which six freezing rates had been applied. The samples were frozen from 20^{o}C to minus 80^{o}C at rates 0.3, 0.6, and 1^{o}C x min^{-1} and to minus 196^{o}C at 30^{o}C x min^{-1}; at rates 10 and 30^{o}C x min^{-1} – from 20^{o}C to minus 196^{o}C; freezing at rate 300^{o}C x min^{-1} was performed by sinking in liquid nitrogen. Ultracryostat ("Nicool"-France), supplied with a writing and programmatic device for running the process, and a low temperature refrigerator ULT 1386-7-V12 ('Rev-co"-USA) were used.

Assay of the concentration of cell suspension and the survival of the strain after preservation. Cell concentration was determined by the indirect method by the limited logarithmic dilutions and 0.1 ml were plated on agar nutrition medium in petri dishes (ø 100 mm). Assessment was made at the 72nd hour of cultivation at 27^{o}C. By the counting petri dishes with above 5 well-formed colonies, but not more than 500 were used. The obtained results were the average sums from the number of the colony forming units (CFU) from three dilutions in three repetitions. Determination of the survival of the strain after preservation was made according to the equation *b/a x 100 = c %,* where *a* is the average number of the colonies before preservation, *b* is the average number of the colonies after preservation and *c* is the survival of the culture in percentage.

Storage. The cryotubes were stored in containers with liquid nitrogen "BT 55" ("Airliquid" – France).

Thawing. Regimes of thawing were at room temperature (20^{o}C) and in a water bath at temperature 27 and 37^{o}C by vigorous shaking of the cryotubes to the thoroughly thawing of the samples.

Results and discussion

Freezing

The cryoprotected samples of the studied brewery strains 179, 181 and 184 were frozen at the indicated six rates. The curves of freezing are showed in Fig. 1.

It was observed, that at rates to 1.0^{o}C x min^{-1} an overcooling in the range 3÷6^{o}C occurred. This assures the subsequent spontaneous crystallization in the whole volume. The last one was detected as a sudden temperature increasing to minus 2^{o}C, a short arrest at this temperature and subsequent uniformly freezing.

After seven days of storage of the described above variants of freezing of the three strains, viability after thawing (37^{o}C) was tested. The results summarizing the influence of the freezing rates on the survival of the strains from species *S. cerevisiae* are shown in Table 1.

For describing the resistance of the yeast strains by this study (Table 1) a curve with the average values of the survival at every applied freezing rate is drawn (Fig. 2).

The results obtained from these experiments showed that more favorable were these rates, at which the cell suspension had gone through an overcooling stage, i.e. these were the slower rates in the range 0.3÷1.0^{o}C x min^{-1}. Survival was the highest by this variant, which had been frozen at rate 0.6^{o}C x min^{-1}, where an overcooling was observed to minus 6^{o}C. In the experiments, by which the cells were frozen at rate 1.0^{o}C x min^{-1} an overcooling to minus 3^{o}C

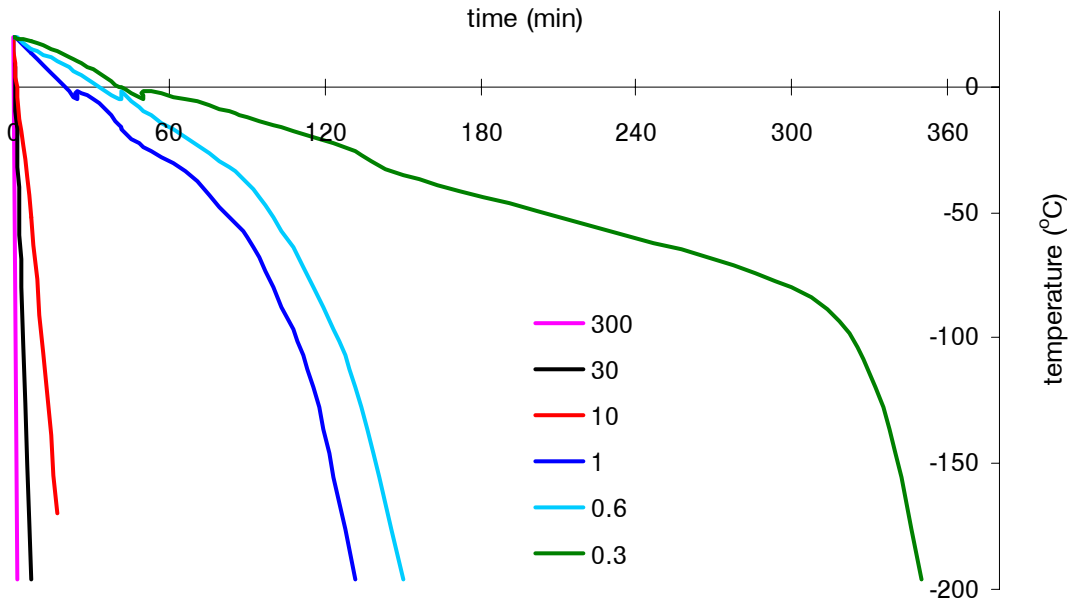

Fig. 1. Freezing rates ($^{o}C \times min^{-1}$).

Table 1. Survival of *S. cerevisiae* strains frozen at different rates.

Freezing rate ($^{o}C \times min^{-1}$)	Strain NBIMCC No	CFU/ml		Survival (%)	
		before freezing	after freezing	of the sample	average value
0.3	179	4.4×10^{8}	2.3×10^{8}	52.27	52.59
	181	4.1×10^{8}	2.2×10^{8}	53.66	
	184	5.4×10^{8}	2.8×10^{8}	51.85	
0.6	179	4.4×10^{8}	2.5×10^{8}	56.85	56.77
	181	4.1×10^{8}	2.3×10^{8}	56.10	
	184	5.4×10^{8}	3.1×10^{8}	57.41	
1.0	179	4.4×10^{8}	2.1×10^{8}	47.73	46.98
	181	4.1×10^{8}	2.0×10^{8}	48.78	
	184	5.4×10^{8}	2.4×10^{8}	44.44	
10	179	4.4×10^{8}	1.5×10^{8}	33.09	33.86
	181	4.1×10^{8}	1.4×10^{8}	34.15	
	184	5.4×10^{8}	6.4×10^{8}	33.33	
30	179	4.4×10^{8}	4.4×10^{7}	14.55	14.38
	181	4.1×10^{8}	5.8×10^{7}	14.15	
	184	5.4×10^{8}	7.8×10^{7}	14.44	
300	179	4.4×10^{8}	4.1×10^{7}	9.32	9.28
	181	4.1×10^{8}	3.8×10^{7}	9.27	
	184	5.4×10^{8}	5.0×10^{7}	9.26	

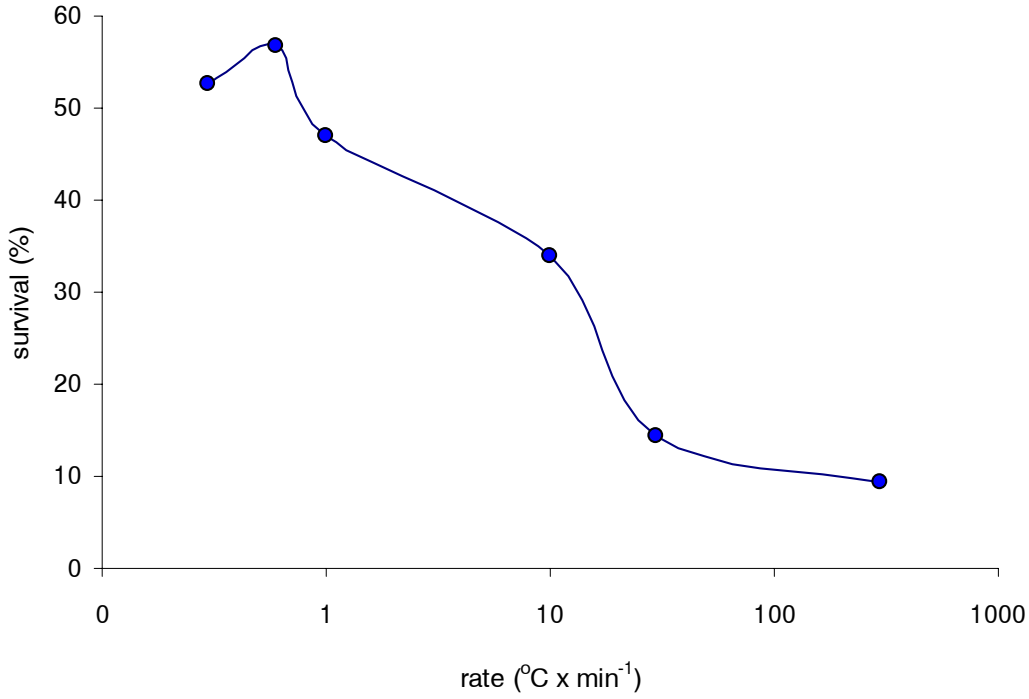

Fig. 2 Influence of the freezing rate on the survival of *S. cerevisiae.*

was detected, followed by crystallization at minus 2°C. We consider that the minimal difference in the survival is due to the different level of ice nuclei forming and their subsequent increasing. By the thawing, at the slowest from the studied rates (0.3°C x min^{-1}), during the fall in the temperature from 20°C to minus 6°C, it was visually established that the cell suspension precipitated in the cryotube. We suppose that this could result in conglomeration of the cells, which later form single colonies. For the collection activities we accepted freezing at rate 0.6°C x min^{-1}.

Thawing

Comparative experiments were carried out with the strains 179, 181 and 184, at freezing rate 0.6°C x min^{-1}. The samples were thawed in three variants.

The achieved by this study data are represented in Table 2. No essential differences regarding the survival of the frozen cells were detected, after thawing in a water bath at temperature 27°C and 37°C. Survival was lower by thawing in air conditions.

The obtained data correspond with the recommended by foreign yeast collections freezing regime [1, 4]. In our work we accept that the samples should be thawed in a water bath at 37°C, by vigorous shaking until thoroughly visual thawing have been observed.

The results from this study are successfully applied by cryogenic preservation of brewery yeast strains from the collection of NBIMCC - freezing at 0.6°C x min^{-1} and thawing in a water bath at a temperature 37°C for 3 minutes.

Table 2. Survival of the three strains *S. cerevisiae*, after thawing at different temperatures

Temperature (°C)	Strain NBIMCC No	CFU/ml		Survival (%)	
		before freezing	after freezing	of the sample	average value
20	179	4.4×10^8	2.1×10^8	47.73	48.22
	181	4.1×10^8	2.0×10^8	48.78	
	184	5.4×10^8	2.6×10^8	48.15	
27	179	4.4×10^8	2.4×10^8	54.55	55.40
	181	4.1×10^8	2.3×10^8	56.10	
	184	5.4×10^8	3.0×10^8	55.56	
37	179	4.4×10^8	2.5×10^8	56.85	56.77
	181	4.1×10^8	2.3×10^8	56.10	
	184	5.4×10^8	3.1×10^8	57.41	

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ВЛИЯНИЕ НА СКОРОСТТА НА ЗАМРАЗЯВАНЕ ВЪРХУ ПРЕЖИВЯЕМОСТТА НА ЩАМОВЕ *SACCHAROMYCES CEREVISIAE* ПРИ КОНСЕРВИРАНЕ

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Резюме

Проведени са опити за определяне на влиянието на скоростта на замразяване върху запазването на жизнеността на пивни дрожди от вид Saccharomyces cerevisiae. Изследвани са шест скорости за замразяване (0.3, 0.6, 1.0, 10, 30 и 300°C x мин$^{-1}$ от 20°C до минус 196°C) и размразяване при три температури – 20, 27 и 37°C. Установено е, че изследваните щамове 179, 181 и 184 запазват жизнеността си максимално при скорост за замразяване 0.6°C x мин$^{-1}$ и размразяване на водна баня с температура 37°C. Резултатите се използват при криогенното консервиране на пивни дрожди в Национална банка за промишлени микроорганизми и клетъчни култури (НБПМКК).