Research of the survival of a consortium of Zygosaccharomyces kombuchaensis yeast and Gluconoacetobacter xylinus bacteria during frozen storage using various protective media

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Abstract. In the present study the survival of the consortium of yeast and bacteria using protective media was investigated. The protective medium consisting of 1 % gelatin, 5 % monosodium glutamate and 5 % sucrose provides the best preservation of living cells of the consortium and allows stabilization of cells for up to 100 days at storage temperatures from -2 ℃ to -10 ℃. The projective medium consisting of 1 % gelatin and 10 % sucrose provides effective storage of the cells at t=-2 ℃ for 100 days; t=-5 ℃ – 80 days; t=-10 ℃ – 60 days. The projective medium of skimmed milk and 7.5 % glucose ensures effective storage of the cells of the consortium at t=-2 ℃ for 100 days; t=-5 ℃ – 80 days; t=-10 ℃ – 80 days. The information obtained is interesting for following researches of the development of technologies for the bioconversion of plant materials.

1 Introduction

The introduction of biotechnologies into the processing of plant raw materials requires solving the issues of maintaining the viability of microbial cells during their storage. The main ways of stabilizing microorganisms during storage are drying and freezing, while one of the significant factors affecting their viability is the composition of the protective medium in which the cells are placed before conservation. The use of protective media containing carbohydrates, amino acids, reconstituted milk, gelatin, and other components reduces the damage to cellular components and increases the guaranteed shelf life of microorganisms [1,2, 9-15].

In the previous studies on the effect of drying parameters and the composition of protective media on the viability of the consortium *Zygosaccharomyces kombuchaensis sp.* yeast and *Gluconoacetobacter xylinus (Brown 1886) Yamada et al 1998* bacteria found [2] that the drying processes are not tolerated by the consortium, the number of viable cells is 15-35 %. The optimal modes are those that provide intensive moisture removal at the very beginning of drying and heating to a temperature not higher than + 32-35 °C throughout the

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entire drying time. To protect the cells of the consortium from the damaging factor of drying and subsequent rehydration, a protective medium consisting of 1 % gelatin, 5 % monosodium glutamate and 5 % sucrose was chosen. It has been established that sucrose penetrates into the cell, creates a high osmotic pressure, dissolves metabolites and prevents the rupture of the cell membrane during drying. The usage of sucrose also contributes to the stabilization of the structure of proteins and a decrease in the temperature of phase transitions of membrane lipids, as well as the formation of hydrogen bonds with polar-charged groups of proteins, stabilizing their structure in the absence of moisture (the "water replacement" hypothesis) [3-16].

Gelatin creates osmotic pressure outside the cell and provides a tight fit of the cell membrane to the plasma during cell rehydration.

The protective effect of monosodium glutamate is associated with its antioxidant activity, the ability to protect cells from free radical oxidation during storage [3, 4].

Freezing is an effective way to stabilize microorganisms. Protective media should protect cells from damage when stored in a frozen state [6-16]. In this regard, it was interesting to choose the optimal composition of the protective medium and frozen storage parameters for the consortium of *Zygosaccharomyces kombuchaensis sp.* yeast and *Gluconoacetobacter xylinus (Brown 1886) Yamada et al 1998* bacteria, used for the bioconversion of plant materials.

2 Materials and methods

Research objects:

- pure cultures of yeast and bacteria that make up a symbiotic culture called SCOBY (symbiotic culture of bacreties and yeasts), adapted and cultivated in the Krasnodar Territory;
- three types of protective media:
- 1% gelatin + 10% sucrose;
- skimmed milk + 7.5% glucose;
- 1% gelatin + 5% monosodium glutamate + 5% sucrose.

Experimental studies were carried out on a set of indicators according to standard methods used in scientific practice.

The assessment of the survival of the consortium under freezing conditions was carried out for 100 days at storage temperatures: t = -2 ℃, t = -5 ℃ and t = -10 ℃. The frequency of cell viability control was on the 30th, 50th, 80th and 100th day. The initial concentration of the consortium was 5×10^{12} CFU/g.

Subsequent reactivation took place in a standard Saburo medium with the addition of peptone and glucose to activate the vital systems of the cells that survived after storage. Next, the cells were seeded on Petri dishes for the standard count of grown colonies.

3 Results and discussion

In the course of the study, the number of surviving cells of the consortium was studied during frozen storage using various protective media.

Table 1 compares the number of viable cells of the consortium after storage for 100 days at t = -2 ℃, t = -5 ℃ and t = -10 ℃ in various protective media, followed by reactivation in a nutrient medium.

Table 1. Percentage of viable cells of yeast and bacteria consortium after storage for 100 days at -2 °C, -5 °C, -10 °C and subsequent reactivation

Researched microorganisms	Shelf life, days	Viable cells, %								
		1 % gelatin + 10 % sucrose			Skim milk + 7.5 % glucose			1 % gelatin + 5 % monosodium glutamate + 5 % sucrose		
		-2 °C	-5 °C	-10 °C	-2 °C	-5 °C	-10 °C	-2 °C	-5 °C	-10 °C
Consortium of *Zygosaccharomyces kombuchaensis sp.* yeast and *Gluconoacetobacter xylinus (Brown 1886) Yamada et al 1998* bacteria	30	98	97	93	98	98	96	100	98	97
	50	95	93	85	97	95	92	99	95	95
	80	93	89	80	95	91	85	99	91	92
	100	90	85	75	91	89	81	98	89	89

When stored in a protective medium of skimmed milk and 7.5 % glucose at -2 °C, -5 °C, -10 °C, the number of surviving cells was accordingly 91 %, 89 %, 81 %.

Due to the fact that the percentage of cell death, equal to 10-15 %, is usually an indicator of the maximum possible number of dead cells of microbiological systems used for subsequent rehydration and activation of microorganisms, it can be concluded that a shelf life of 100 days, followed by effective activation microorganisms at storage temperatures of -2 °C, -5 °C, -10 °C can ensure the usage of a protective medium consisting of 1 % gelatin, 5 % monosodium glutamate and 5 % sucrose.

The protective medium, consisting of 1 % gelatin and 10 % sucrose, ensures efficient storage of the cells of the studied consortium at -2 °C for 100 days; at t = -5 °C – within 80 days; at t = -10 °C – within 60 days.

The protective medium, consisting of skimmed milk and 7.5 % glucose, provides effective storage of the cells of the consortium under study at a temperature of -2 °C for 100 days; at t = -5 °C – within 80 days; at t = -10 °C – within 80 days.

Figure 1 shows a comparison of the number of living cells of the studied consortium of yeast and bacteria after storage for 100 days at t = -2 °C, t = -5 °C and t = -10 °C and subsequent reactivation using various protective media.

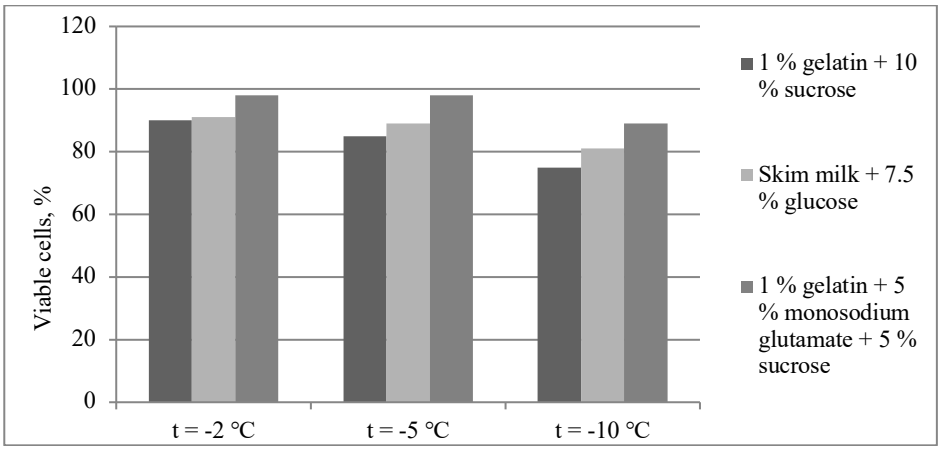

Fig. 1. The number of living cells (%) of the studied consortium of yeast and bacteria after storage for 100 days at t = -2 °C, t = -5 °C and t = -10 °C and subsequent reactivation

Analysis of the data presented in the figure made it possible to conclude that the protective medium consisting of 1 % gelatin, 5 % monosodium glutamate and 5 % sucrose showed the highest percentage of surviving cells over 100 days of storage at temperatures of -2 °C, -5 °C, -10 °C – the number of surviving cells was – 98, 98, 89 %, accordingly.

The percentage of two other protective media was worse. By the end of storage of the studied consortium with a protective medium of 1 % gelatin and 10 % sucrose at temperatures of -2 °C, -5 °C, -10 °C, the number of surviving cells was accordingly 90, 85, 75 %.

Thus, our recommendation for the storage of the studied consortium of microorganisms is the usage of a protective medium consisting of 1 % gelatin, 5 % monosodium glutamate and 5 % sucrose. Storage is possible at temperatures down to -10 °C for 100 days.

4 Conclusion

In the present study, the survival of the consortium of *Zygosaccharomyces kombuchaensis sp.* yeast and the *Gluconoacetobacter xylinus (Brown 1886) Yamada et al 1998* bacteria during frozen storage using protective media was carried out. It has been established that a protective medium consisting of 1 % gelatin, 5 % monosodium glutamate and 5 % sucrose provides the best preservation of living cells of the consortium and allows stabilization of cells for up to 100 days at storage temperatures from -2 °C to -10 °C.

The protective medium, consisting of 1 % gelatin and 10 % sucrose, ensures efficient storage of the cells of the studied consortium at -2 °C for 100 days; at t = -5 °C – within 80 days; at t = -10 °C – within 60 days. The projective medium, consisting of skimmed milk and 7.5 % glucose, provides efficient storage of the cells of the consortium at a temperature of -2 °C for 100 days; at t = -5 °C – within 80 days; at t = -10 °C – within 80 days. The data obtained are interesting for future researches on the technologies for the bioconversion of plant materials.

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