

## AN INVESTIGATION OF THE TREHALOSE ACCUMULATION DYNAMICS BY YEASTS FROM GENUS *SACCHAROMYCES*

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### Summary

*The trehalose accumulation by 77 superficially cultivated yeast strains from genus Saccharomyces was investigated in dynamics. The conditions were determined for the maximal achievement of the storage carbohydrate in the cells. Experiments with optimal duration of 120 - 144 hours were performed on two nutrient media. The brewery yeasts produced significantly more trehalose (10 - 13%) when compared with the wine and baking ones.*

### Introduction

The trehalose accumulation capability (10 - 15%) of the yeasts from genus *Saccharomyces*, depending on the culture's age and the cultivation conditions, is well-known [7, 8, 9]. This storage carbohydrate plays the role of an osmotic regulator when using nutrient media with raised pressure [3]. The mentioned physiologic function of the trehalose is connected also with the increase of the cryo-resistance of these micro-organisms when preserving them at low

temperatures and lyophilization [1, 2, 5, 10]. Because of this, investigations that have to do with the conditions for increase of the trehalose concentration in the cell are essential for saving the viability and the characteristics of the mentioned strains after conservation.

The aim of the present work is to study in dynamics, the trehalose content by 77 yeast strains from genus *Saccharomyces*, at superficial cultivation, routinely used in their preparation for long-term preservation.

### Materials and Methods

**Microorganisms.** Investigations were performed on 77 industrial and collection strains from the National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC): 7 brewery, 9 wine, 21 baking Bulgarian cultures, and 40 foreign collections' strains, finding application in the brewery, wine and baking industry. Representatives of the

species: *Saccharomyces cerevisiae* (NBIMCC № 457 - 461, 463, 464, 537, 541, 569 - 588, 605, 1212, 1276 - 1279, 1448 - 1450, 1452, 1453, 1516, 1564 - 1566, 1809 - 1813, 1978, 1991, 2148 - 2151), *S. uvarum* (NBIMCC № 177, 179 - 182, 184, 185, 543, 1082, 1083, 1084), *S. bayanus* (NBIMCC № 456, 462 and 1980),

*S. vini* (NBIMCC № 545), *S. paradoxus* (NBIMCC № 1981), *S. diastaticus* (NBIMCC № 802), *S. coreanus* (NBIMCC № 1081), *S. oviformis* (NBIMCC № 895) *S. pastorianus* (NBIMCC № 82 and 1979), *S. carlsbergensis* (NBIMCC № 1517), were included.

**Nutrient media.** The investigations were performed on two nutrient media - beer agar (BA) and malt agar (MA) [4]. The cultures

were incubated during six days at 30°C.

**Determination of the trehalose quantity.** The method for carbohydrate determination after Traveyan and Harrison [14] was applied on every 24 hours from the second until the sixth day of cultivation. The biomass concentration was determined by drying at 105°C until constant weight was reached.

## Results and Discussion

The trehalose quantity varied depending on the species, the conditions of growth and the age of the cultures, and could reach 20% of the cells' dry content [7, 12, 13]. The formation of the disaccharide started after the sixth hour of cultivation, as its accumulation was quicker at the beginning of the logarithmic phase [11].

Different duration experiments were performed by the use of superficial seeding on BA and MA for determination of the optimal incubation continuance. A representative extract of a part of the studied strains, generalising the different yeast groups, was accomplished (Table1).

Regarding the used nutrient media, it was found out that the MA was better for the trehalose formation. An increased content of the disaccharide in the yeast cells was registered when cultivating on MA. Many of the investigated strains accumulated the storage carbohydrate up to a twice-higher quantity when compared with the variant where the growth is realised on BA.

Regarding the incubation continuance, it was obvious that the cultures showed maximal trehalose production capability for 5 - 6 days, while during the last day the level was retained or insignificantly lowered.

Following the dynamics of the trehalose accumulation, it was found out that this process was slower when superficial cultivation had been performed. At the same

time the achieved results gave a precondition for an increase of the survival ratio of the yeast strains after conservation and lyophilization.

For determination of the quantity of produced trehalose, among all the investigated yeasts an attention deserved the collection strains with accumulation of over 8% compared to the dry substance, i. e.: brewery, with catalogue numbers 1810 - 13%, 1809 - 10.1%, 1811 - 9.3%, 1813 - 10.5%; baking cultures - 576 - 8.6%, 577 - 10.9%, 588 - 9.8%, 1279 - 8.1%; wine yeasts - 463 - 8.6%, 1812 - 9.5%; collection - 2151 - 9.8%, etc.

The representatives of the *S. cerevisiae* species were with the highest percentage of trehalose in the cells - 8 to 13%. The storage carbohydrate was in significantly lower quantities by the other species. Thus by *S. pastorianus* 1979 it was 4.0%, by *S. paradoxus* 1981 - 4.2%, by *S. vini* 545 - 7.7%, by *S. diastaticus* 802 - 2.8%, by *S. oviformis* - 2.5%, etc.

As a result from the screening for trehalose content of 77 strains from genus *Saccharomyces*, we could say that the produced quantity was higher (over 10%) by the brewery yeasts when compared with the rest of the groups.

Thus the achieved results give us a reason to overlook the accepted for routine in our work conditions for propagation, preparation and conservation of the yeasts from genus *Saccharomyces* [6].

Table 1. Dynamics of the trehalose accumulation by strains *S. cerevisiae*.

NBIMCC №	Continuance of the cultivation on							
	BA	MA	BA	MA	BA	MA	BA	MA
	48 hours		96 hours		120 hours		144 hours	
	Trehalose content (%)							
460	2.5	4.4	3.5	5.8	3.8	6.9	3.8	7.0
463	2.8	5.1	3.7	7.7	3.6	8.6	3.6	8.6
576	3.3	5.8	5.3	7.5	5.9	8.7	5.8	8.6
577	2.4	6.2	5.1	8.7	5.4	10.9	5.4	10.9
579	3.6	4.2	4.0	5.2	5.3	7.6	5.4	7.6
581	3.8	4.2	4.1	4.9	4.3	9.6	4.3	9.5
587	3.6	6.5	3.9	8.0	5.4	10.1	5.5	10.0
588	2.4	4.0	4.7	6.6	5.3	9.9	5.2	9.8
1450	2.2	2.8	2.5	6.9	3.5	8.8	3.7	8.9
1565	3.0	4.1	3.8	4.5	4.4	8.4	4.6	8.5
1809	2.7	5.6	3.8	7.6	4.6	10.1	4.8	10.1
1810	2.6	5.5	2.8	7.8	4.2	1.9	4.1	13.0
1811	3.8	6.6	4.6	8.8	4.9	9.3	4.9	9.3
1813	4.8	6.0	6.4	6.4	8.5	1.7	8.5	10.5
2151	3.9	5.5	5.5	6.9	7.7	9.9	7.8	9.8

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