STUDIES CONCERNING THE BIOSYNTHESIS OF CARBOHYDRATES BY YEAST STRAINS BELONGING TO THE SACCHAROMYCES GENUS, ISOLATED FROM DIRECTLY PRODUCING GRAPEVINES

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Abstract

The purpose of this study is the isolation of wine yeasts with high carbohydrate synthesis potential from wine sediments obtained from direct producer hybrids. Maximum productivity of yeast strains was observed in the medium in which glucose was substituted by molasses, the increase in biomass varying, depending on the concentration used, from 4.9 to 6.9 g/l, these values being 194% and 276% respectively larger than the controls'.

Key words: wine yeasts, carbohydrates, mannan, culture media.

INTRODUCTION

Presently there is an exceptional interest towards natural bioactive polysaccharides due to their numerous applications in the most diverse fields. Polysaccharides were studied and used in pharmaceutical purposes because of their high biologic activity, including anti-carcinogenic and immunomodulator effects. (Dritz, S. S., et al 1995, Mao; X. F et al 2005; Tokunaka et al, 2000 ;Bohn & Bemiller, 1995 ; Gomez-Verduzco G., et al. 2009, Joaquin Perez-Guisado, 2007). This attention is especially focused on levurian polysaccharides.

The type of mannan formed by levurian polysaccharides is one of the characteristics of the species and can be used in identifying and classifying them. The immunologic characteristics of yeasts depend largely on the nature of mannans. Certain yeast mannans can be produced with increased yield and can be used as thickening agents, dispersion agents and in other similar applications. (J. F. T. Spencer, P. A. J. Gorin A, 1973).

One can find multiple types of sugars in the cellular membrane of yeasts, out of which the fundamental types are the mannan linked to the cellular membrane proteins and the glucan. β-glucans are part of the structure of the internal layer of the cell membrane, while the outer layer is formed by manan. (S. De Baets and E.J. Vandamme, 2001).

Polysaccharides produces by levurians have been grouped into endopolysaccharides (PPS) or exopolysaccharides (EPS). (Cheng, Y. H., D. N. Lee ; A.R. El –SHAMY and Nehad E.A., 2010) Exopolysaccharides

have a high molecular weight (monosaccharide polymers) (>20) and are secreted by microorganisms into the surrounding environment.

Chromatographic analysis of hydrolyzed intracellular polysaccharides indicated a glucose: manose: galactose: xilose ratio of 1: 1.32: 1.07: 0.35. (Jwanny, E. W. and Rashad, M. M. 1983)

The synthesis of levurian polysaccharides is influenced by the constituents of the nurturing environment (the nature of C, N and P sources and their concentration, the C/N ratio, metallic ions), physical-chemical factors (temperature, pH, aeration level), and the duration of the periodical cultivation cycle. Depending on the producer's cultivation condition, the chemical composition of the polysaccharides and their molecular mass changes, as well as the quantitative content of their components, which has a direct influence on their viscosity, a parameter which determines their practical importance to a great degree. (Fang Q. H., Zhong J. J, 2002)

There are numerous studies concerning the synthesis of carbohydrates by microorganisms, but the issue of selecting strains with increased activity remains current, being linked to both the characteristic production instability of microorganisms and the lack of culturing mediums and orientated synthesis procedures, specific to the producer.

High-performance carbohydrate producers are yeasts of the Saccharomyces genus (Gonzalez-Ramos D., Cebollero E., Gonzalez R. A., 2008), and their activity can be substantially upgraded by the modification of the nutrition factors of cultivation parameters and of stimulators and regulators. (Stehlik-Tomas V., et al, 2004, Kiran M. Desai, et al, 2005)

In this context, the necessity for investigations in order to select the strains with high biosynthetic ability, the clear definition of the optimal composition of the culture medium and the establishment of optimal cultivation parameters becomes paramount. From this perspective, yeasts belonging to the Saccharomyces genus - confirmed producers of bioactive carbohydrates - show great potential.

The purpose of this study is the isolation of wine yeasts with high carbohydrate synthesis potential from wine sediments obtained from direct producer hybrids. We also studied the physiological and biochemical characteristics of the isolated strains.

MATERIALS AND METHOD

Our objects of study were Saccharomyces cerevisiae yeast strains, isolated in unmixed culture, from two types of direct-producer hybrids: Noah (white grapes) and Othella (red grapes.

Noah (Nova, white grape)

The grape bunches are cylindrical or conical in shape, of intermediate size, with round grapes of white-green hue, fleshy pulp and strawberry aroma. They shed easily, which makes harvesting them more difficult. Due to their thick skin and mucilaginous interior, they are difficult to turn into wine.

Othella (black grape)

Grape bunches are large, winged, with large, black, fleshy grapes, with a strawberry aroma. The strains were isolated by seeding them in multiple stages on different liquid and agarized mediums.

The culture mediums used were:

- Rieder medium: 30.0 g/l glucose, 3.0 g/ l $(NH_4)2SO_4$, 0,7 g/l $MgSO_4 \bullet 7H_2O$, 0,5 g/l NaCl, 0,4 g/l ,$Ca(NO_3)2$, 1,0 g/l KH_2PO_4, 10 ml yeast autolyze, 1 l potable water, pH 5.0-6.0
- Malt stum - 30g malt extract to 1000/ml water
- YPD medium: 2% yeast extract, 2% peptone, 3% glucose.

The isolated Noah hybrid strain was marked as NA and the isolated Othella hybrid strain was marked as OR. The determination of productivity and dry mass of these strains was conducted gravimetrically.

The determination of the number of cells to liquid volume ration was performed with a DEN 1 densiometer. The carbohydrate content in yeast biomass and culture liquid was performed spectrophotometrically according to the following protocol: a clear, aqueous carbohydrate sollution was placed in a test tube, to which sulphuric acid and phenol are added. The sollution gains a yellow-orange hue, as a result of the interaction between sugars and phenol. Absorbancy at 420 nm is directly proportional to the initial concentration of carbohydrates in the sample.

The sulphuric acid makes all the non-reducing sugars convert to reducing sugars, so this method determines the total sugar content.

The method is non-stoichemetric and thus requires the preparation of a calibration curve, utilizing a series of standard carbohydrates of known concentration.

Carbohydrate extration by friction was performed according to protocol described in specialty literature, based on utilizing acid and alcaline sollutions. (Rudic V., s.a., 2003)

In order to interpret the obtained data regarding number and lenght of scions, the ANOVA (variation analisys) test was used.

OBTAINED RESULTS

Through multiple passages on agarized mediums, we isolated four colonies of well-developed yeasts, from red wine, and four out of white

whine, marked as OR and NA, from 1 to 4. Culture characteristics of these yeasts vary greatly amongst themselves, according to their origin.

Morpho-cultural characteristics were studied on selected strains, and the carbohydrate content was determined by cultivation of agarized malt stum at +28°C for 72 hours.

Results are shown in Table 1.

Table 1

Morphological characteristics and carbohydrate synthesis of selected strains

No.	Yeast strains	Colony type and diameter	Colony consistency	Colony color, hue	Carbohydrates, % D.M.
1	OR 1	S-ø 3.5-4 mm	Pasty, mate	White	12.7
2	OR 2	R-ø 4 mm	Glossy	Cream-colored	13.4
3	OR 3	R-ø 4.3mm	Mucilaginous	Cream-yellowy hue	28.2
4	OR 4	S-ø 3.7mm	Mucilaginous	White	24.8
5	NA 1	S-ø 4.2 mm	Mate, pasty	Cream-colored	25.0
6	NA 2	S-ø 5mm	Glosssy	White-yellowy	15.8
7	NA 3	S-ø3.9 mm	Glossy	Cream-colored	26.0
8	NA 4	R-ø4.3 mm	Pasty	White	16.2

In similar culturing condition, the strains synthesize different quantities of carbohydrates, with values ranging from 12.7% - 28.2 % D.M. Of the tested strains, the best results relative to carbohydrate content can be observed in the case of NA3 (26% carbohydrates in dry mass) and OR3 (28.2%).

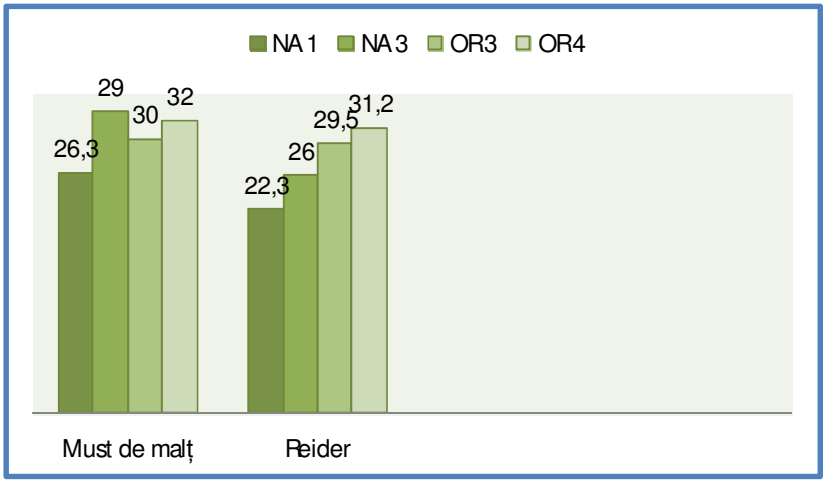

Fig. 1 Dry biomass production (g/l) in 4 of the selected strains, on 2 culture environment variants Maximum values were determined for OR3 and OR4 strains on both culture medium variants.

Table 2

Productivity, quantitative and qualitative carbohydrate content in selected strains, cultivated on liquid mediums

Determined factor	NA 1 strain		NA 3 strain		OR 3 strain		OR 4 strain	
	Rieder medium	YPD medium	Rieder medium	YPD medium	Rieder medium	YPD medium	Rieder medium	PD medium
BU, g/l	3.6	6.3	3.1	6.8	3.2	5.8	3.3	3.1
Carbohydrates, % D.M	22.3	20.8	26.0	24.0	29.5	26.7	28.2	26.2
Solluble fraction in H_2O(mono-, disaccharides), % D.M.	1.1	0.4	1.7	0.5	1.4	1.1	1.6	1.8
Solluble fraction in NaOH 3% (mananproteins), % D.M.	3.7	0.9	5.4	0.7	4.1	4.0	3.7	1.7
Solluble fraction in H_2SO_4 2% (glycogen type), % D.M.	1.6	0.3	1.3	0.4	1.9	1.6	1.2	1.0
Insolluble fraction in alcaloids and acids (β-glucans), % D.M.	21.8	15.3	20.2	11.8	16.4	16	14.5	13.6
Fraction Σ, % D.M.	28.2	16.9	28.6	13.4	23.8	23.0	22.7	18.1

From the analysis of the obtained data we found that the carbon sources present in the nurturing evironment (Table 3) show selective effects on the multiplication and synthesis of carbohydrates in studied strains. Maximum yeast productivity was recorded in the medium in which glucose was substituted by molasses, the biomass increase varying, depending on the concentration used, from 4.9 to 6.9 g/l, these values being 194% and 276% respectively larger than the control sample's and being distinctly significant. While estimating the carbohydrate synthesis activity, we concluded that the subtitution of glucose with fructose leads to an increase in carbohydrate content in yeasts. Differences compared to control samples are distinctly significant.

Table 3

The carbon source influence on the multiplication and synthesis of carbohydrates by *Saccharomzces. cerevisiae* NA 1 yeast strain

Carbon source	Carbon source conc. g/l	Number of cells, 1x 10^6 ml^{-1}	% Control	Prod. BU g/l	% Control	Carbohydrates % D.M.	% Control
Glucose	20	12.7	104.09	2.8	112.0	26.4	102.32
	40	14	114.75	3	120.0	34.2	132.55
Sucrose	20	12.4	101.63	2.5	100.0	28.6	110.85
	40	14.5	118.85	3	120.0	35.5	137.59
Fructose	20	12.8	104.91	2.8	112.0	35.2	136.43
	40	16.5	135.24	3.4	136.0	38.5	149.22
Molasses	20	25	204.9	4.9	194.0	32	124.03
	40	29.3	240.16	6.9	276.0	33.6	130.32
Reider Control	20	12.2	100	2.5	100	25.8	100

STUDY CONCLUSIONS

Under similar cultivation conditions, isolated strains synthesise different quantities of carbohydrates. Selected wine sediment yeast strain productivity is largest on the malt stum medium, proving superior to the Reider medium due to the complex composition. Maximum productivity of yeast strains was observed in the medium in which glucose was substituted by molasses, the increase in biomass varying, depending on the concentration used, from 4.9 to 6.9 g/l, these values being 194% and 276% respectively larger than the controls'.

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