PHENOTYPICAL SIGNS AND CHEMICAL COMPOSITION OF *Saccharomyces cerevisiae* – MANNOPROTEIN PRODUCERS

Agafia USATÎI[*], Elena MOLODOI[*], Nadejda EFREMOVA[*], Natalia CHISELIŢA[*], Tamara BORISOVA[*], Ludmila FULGA[*]

[*] *Institute of Microbiology and Biotechnology of Academy of Sciences of Moldova, Chişinău, Moldova*
Corresponding author: Agafia Usatîi, Institute of Microbiology and Biotechnology, Academiei Street, no. 1, Chishinau, Moldova
phone:+373(22)738013, fax: +373(22)725754, e-mail: usatyi.agafia@gmail.com; biotehnol_asm@mail.ru

Abstract. Phenotypical signs and chemical composition of *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-19 yeast strains are described in this article. The presence of protein complexes with high content of irreplaceable amino acids and antioxidant enzymes, as well as polysaccharides with predominance of mannoproteins allow to recommend these yeast strains for the utilization in biotechnology. Results are of interest for the standard description of yeast strains offered as object for industrial appointment.

Keywords: *Saccharomyces cerevisiae,* mannoproteins, yeast, phenotypical signs, chemical composition, polysaccharides, β-glucanes, lipids, superoxide dismutase, catalase

INTRODUCTION

The utilization of yeast strains with high biotechnological potential presents great interest for economic security. The major components of yeast cell walls are polysaccharide components which have an important role in maintaining of the permeability properties. The investigation in this field have relevance for directed synthesis of cell wall polysaccharides.

Yeast cell is surrounded be a membrane which are 25-30% of the dry weight of the cell. The chemical composition of cell wall of *Saccharomyces* yeast strains is formed by 85...90% of polysaccharides and 10...15% of proteins [8,29]. Cell wall polysaccharides consist of water-soluble mannanes, alkali-soluble glucanes, alkali-insoluble glucanes and chitin [11,17]. The mannoprotein consists of a major polysaccharide and a minor protein part. Studies on the localization of mannoproteins in the cell walls of various strains of yeasts have demonstrated that they interact with phosphates, piruvates, glucuronic acids and other [7,15].

The proportion and composition of polysaccharides is highly dependent upon the strain of yeast. Aguilar-Uscanga has mentioned that *Saccharomyces cerevisiae* contained glucanes ranging 71.4...127.4 μg mg^{-1}, mannanes – 86.5...93.3 μg mg^{-1} dry weight and chitin – 2.4...6.2% cell wall [2].

There is increasing commercial interest in the polysaccharides of yeast cell walls which can be used in food, pharmaceutical industry and cosmetology. Only mannoproteins and glucans of *Saccharomyces cerevisiae* yeast strains have been considered in the context of these applications.

Due to the most important characteristics such as high water solubility, relative low molecular weight (15-30 kDa), antioxidant properties mannanes isolated from yeast cell walls can be considered as natural potential protector [21]. Mannoproteins can be proposed for wine stability [12,13], for medicine as immunomodulatory [18,24] and antimutagen remedy [22].

Yeast mannoproteins have a variable ability to reduce toxic effects of aflatoxins, that are one of the most dangerous groups of mycotoxins present in food or feed. Aflatoxins are the most toxic naturally known occurring carcinogens [27]. The protective mechanism of action of mannoproteins is based on the ability to reduce DNA damage induced by aflatoxins through formation of a supramolecular complex between toxins and mannoproteins [28].

The analysis of application areas and the mechanism of action of microbial polysaccharides have demonstrated the importance of studies of technological properties of yeast strains selected for industrial production. In this context investigations of biochemical composition of yeast have a great practical value and contribute to the improvement of biosynthetical potential of yeast strains producers of mannoproteins.

MATERIALS AND METHODS

Strains. Yeast strains *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-19 previously selected as a source of mannoproteins [34]. Morphological, cultural and physiological parameters of yeast strains were established according to [4, 20].

Medium. The following medium for were used in this study: seed culture medium with beer wort, fermentation medium YPD, (w/v), (g L^{-1}): glucose - 20.0, peptone - 20.0, yeast extract - 10.0; Rieder, (w/v), (g/L^{-1}): glucose - 30.0, $(NH_4)_2SO_4$ – 3.0, $MgSO_4 \cdot 7H_2O$ - 0.7, NaCl - 0.5, $Ca(NO_3)_2$. 0.4, KH_2PO_4 – 1.0, yeast autolysate – 10.0, pH 5.0-6.0 [3].

Culture conditions. Cultivation was carried out in Erlenmayer 1 L^{-1} fkasks containing 0.2 L^{-1} of the culture medium at 200 rpm agitation rate, 25±1ºC, with air flow rate 7-8 mg L^{-1} for 96 hours.

Biomass was determined gravimetrically [23]. Protein content was determined spectrophotometrically by Lowry [25]. The determination of total content of carbohydrates was effectuated according to spectrophotometrically method using antron reagent,

standard-D-glucose [9, 10]. Measurements were performed using T60 UV/VIS spectrophotometer [33, 35]. Lipid content was determined by the method Bligh, Dyer in modification of Kates [16] and by the new procedure [5]. Amino acids composition was identified using analyzer AAA-339 „Microtehnica" [29]. Fractional composition of lipids was determined by thin layer chromatography and densitometry [30]. Fatty acid composition was determined as the methyl esters of fatty acids by gas-liquid chromatography of fatty acid using Chrome-5 chromatographer [32]. The determination of catalase activity was effectuated according to [1]. The determination of activity of superoxide dismutase was carried out according to the method proposed by were performed using *t* test. A value of $P<0.05$ was considered significant. The results were performed according to [6].

Statistical analysis: Results were expressed as the mean±standard deviation (SD). Statistical evaluations Student's t-test.

RESULTS

Biosynthetical potential of yeasts is associated with the metabolic flexibility. The study of biological peculiarities of yeasts would contribute to the realization of processes of directed synthesis in necessary direction. In this context the study of morpho-cultural and physiologo-biochemical parameters of yeast strains producers of bioactive substances is of great value.

The study of *Saccharomyces cerevisiae* CNMN-Y-18 yeast strain and *Saccharomyces cerevisiae* CNMN-Y-19 yeast strain cultivated on YPD or Rieder medium has demonstrated that cells have cylindrical or ellipsoidal form, are single or in pairs and rarely form agglomerations. The size of cells is typically 5-10 μ in diameter. Can multiply either asexually by vegetative multiplication by budding or sexually by forming ascospores. Sometimes, this yeast strain can form pseudohyphae. The type of respiration is aerobic. Yeast strain can form sediment in liquid culture medium. It is non-pathogenic microorganism.

Saccharomyces cerevisiae CNMN-Y-18 yeast strain has S-form colonies with diameter of 1-2...3-6 mm at the cultivation on beer wort and Rieder medium. This yeast strain has S- and R-form colonies at the cultivation on YPD medium. Colonies appear white, cream and fairly circular in shape (Fig. 1).

Among physiologo-biochemical parameters of the *Saccharomyces cerevisiae* CNMN-Y-18 yeast strain we have mentioned the fermentation capacity, the capacity of the assimilation of glucose, maltose, zaharose, fructose, galactose, raffinose, arabinose, xylose, glycerin, the alcohol etilic. Does not assimilate D-lactose, L-ramnose, sorbitol, D-trehalose, inozitol, dulcit, melibiose. Of the range of nitrogen sources this yeast strain assimilates ammonium sulfate and ammonium phosphate.

Does not assimilate nitrates, urease, lysine.

Content of dry biomass of *Saccharomyces cerevisiae* CNMN-Y-18 yeast strain varies from 3.1 g/L (nutritive medium Rieder) to 6.18 g/L (nutritive medium YPD).

Saccharomyces cerevisiae CNMN-Y-19 yeast strain has S-form colonies with diameter of 1-2...3-6 mm at the cultivation on beer wort, YPD and Rieder medium. Most yeast colonies appear white, cream, or pink in color (Fig. 2).

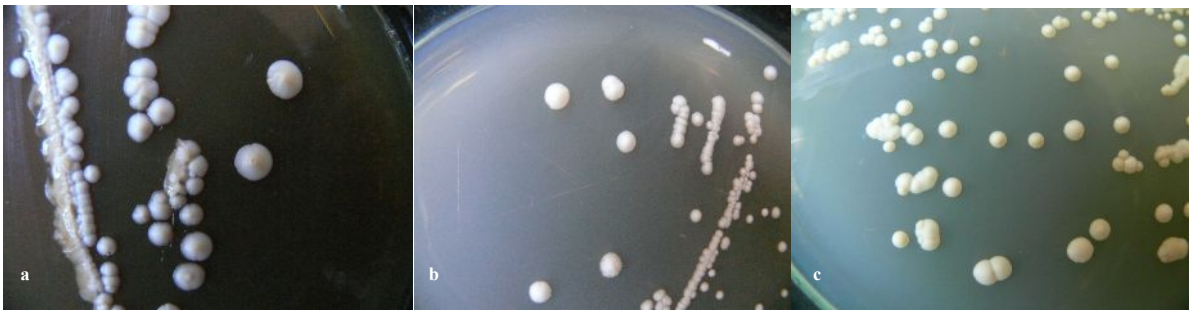

Figure 1. Yeast colonies of *Saccharomyces cerevisiae* CNMN-Y-18 cultivated at different mediums of culture. **a.** Beer wort; **b.** YPD; **c.** Rieder

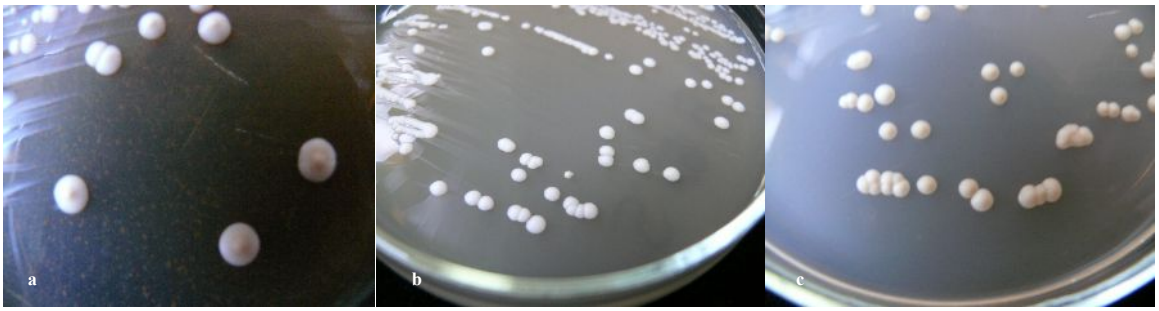

Figure 2. Yeast colonies of *Saccharomyces cerevisiae* CNMN-Y-19 cultivated at different mediums of culture. **a.** Beer wort; **b.** YPD; **c.** Rieder

Among physiologo-biochemical parameters of the *Saccharomyces cerevisiae* CNMN-Y-19 yeast strain we have mentioned the fermentation capacity, the capacity of the assimilation of glucose, maltose, zaharose, fructose, galactose, raffinose, arabinose, xylose, glycerin, the alcohol etilic. They do not assimilate D-lactose, L-ramnose, sorbitol, D-trehalose, inozitol, dulcit, melibiose. Of the range of nitrogen sources this yeast strain assimilates ammonium sulfate and ammonium phosphate. They do not assimilate nitrates, urease, lysine.

Saccharomyces cerevisiae CNMN-Y-19 yeast strain at submerged cultivation on YPD medium accumulated 3.36 – 5.96 g/L dry biomass and 2.66 – 2.79 g/L dry biomass on medium Rieder.

Research on chemical composition of selected as producers of mannoproteins species of yeasts have established a moderate protein content from 28.76 to 31.09% of dry biomass for *Saccharomyces cerevisiae* CNMN-Y-18 and from 31.76 to 36.53% of dry biomass for *Saccharomyces cerevisiae* CNMN-Y-19 yeast strain (Fig. 3). Determination of lipid contents have demonstrated that dry biomass of both yeast strains contains from 6.2 to 6.6%. The carbohydrate content in the biomass of *Saccharomyces cerevisiae* CNMN-Y-18 vary within the limits of 34.02 (YPD) and 35.58% (Rieder), as well as 30.17 (YPD) and 33.9% (Rieder) for *Saccharomyces cerevisiae* CNMN-Y-19.

The study of amino acid composition have established high content of essential amino acids – valine, leucine, lysine,arginine for both yeast strains which can serve as source of protein (Fig. 4).

An important role in protection from oxidative stress have antioxidant enzymes superoxide dismutase and catalase.

The obtained results affirmed that the activity of catalase in *Saccharomyces cerevisiae* CNMN-Y-18 at the cultivation on nutrient mediums YPD and Rieder was 2035 and 2391 U/mg protein, respectively and the activity of superoxide dismutase was 153...168 U/mg protein. For *Saccharomyces cerevisiae* CNMN-Y-19 strain catalase activity was 2709 and 2495 U/mg protein, respectively, superoxide dismutase activity reached 227 and 240 U/mg protein. Therefore, obtained data have demonstrated that selected yeast strains can serve as a source of high antioxidant activity bioproducts (Table 1). According to the authors, activity of catalase enzyme in the *Candida boidinii* wild yeast strain was 2530 U/mg of protein [14]. Superoxide dismutase activity in *Saccharomyces cerevisiae* wild strains grown in glucose and ethanol was 58.5 and 165 U/mg of protein, respectively [26].

The study of fractional composition of lipids extracted from studied yeast strains established the following bioactive principles – phospholipids, sterols, free fatty acids, mono-, di- and triglycerides. According to the data presented in Fig. 5, we can affirm that for yeasts is a specific high content of sterols (provitamine D) up to 14.33% of tota lipids.

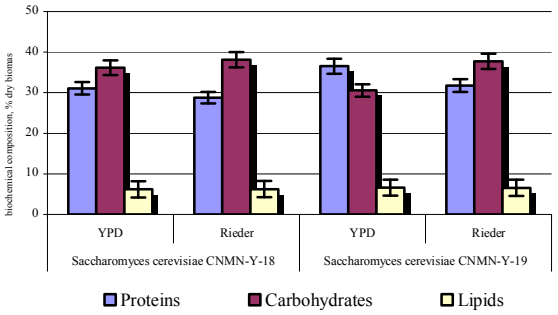

Figure 3. Chemical composition of *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-19 yeast strains

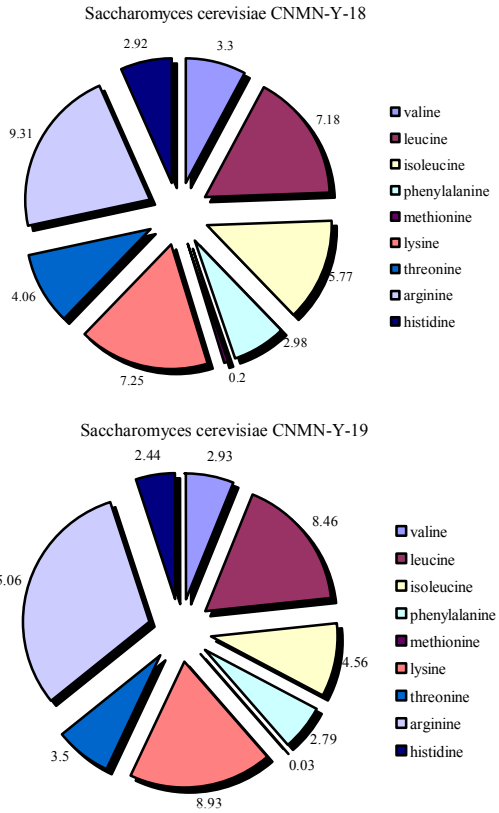

Figure 4. Amino acids composition of yeast strains, % of total identified

The phospholipid content was 3.67 – 4.45% of the sum of the total lipid fractions. Content of free fat acids varies in the range of 4.36 – 8.58%, mono and diglycerides from 10,23 to 15.55%, triglicerides from 17.38 to 22.11% of the sum of the total lipid fractions.

Therefore the composition of lipids synthesized by yeast is particularly valuable as a source for emulgators because they contain organic compounds, especially phospholipids, forming stable emulsions.

Research carried out on fatty acid spectrum has demonstrated a valuable composition due to the presence of essential fatty acids such as linoleic 11.05% (*Saccharomyces cerevisiae* CNMN-Y-18) and 3.72 % (*Saccharomyces cerevisiae* CNMN-Y-19) and linolenic 2.21 and 2.03% respectively (Fig. 6). It is important to note that content of saturated fatty acids vary from 44.04%-39.06% and that of unsaturated

Table 1. Catalase and superoxide dismutase activity at *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-19

Enzymes	*Saccharomyces cerevisiae* CNMN-Y-18		*Saccharomyces cerevisiae* CNMN-Y-19	
	YPD	Rieder	YPD	Rieder
Catalase, U/mg protein	2391±102	2035±62	2709±85	2495±102
Superoxide dismutase, U/mg protein	168±9.43	153±7.80	240±9.92	227±10.43

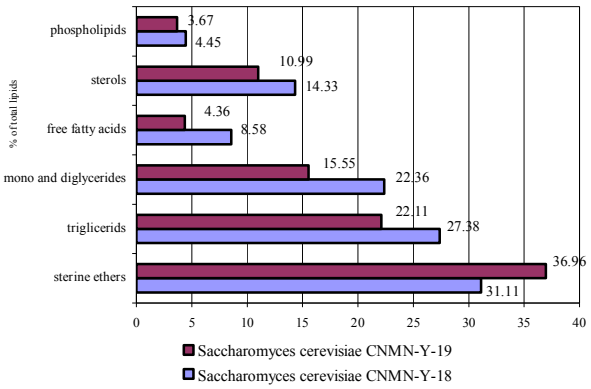

Figure 5. The fractional composition of lipids at *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-19

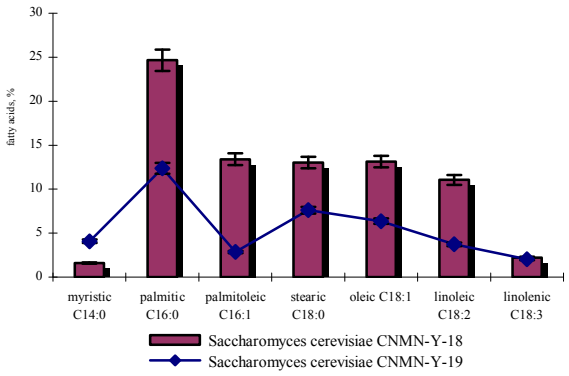

Figure 6. The content of fatty acids in *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-19

Table 2. The content of β-glucanes and mannoproteins at *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-19 yeast strains

Polysaccharides	*Saccharomyces cerevisiae* CNMN-Y-18		*Saccharomyces cerevisiae* CNMN-Y-19	
	Medium YPD	Medium Rieder	Medium YPD	Medium Rieder
β – glucanes, % dry cell weight	17.41±0.01	13.50±0.21	16.23±0.16	16.70±0.18
Mannoproteins, % dry cell weight	10.93±0.14	11.60±0.11	7.09±0.77	6.80±0.01

acids constitute 55.96-69.51%. The predominance of unsaturated fatty acids indicates real possibilities for the use of yeasts as a food supplement to prevent different pathological disorders.

Such polysaccharides as β-glucanes and mannoproteins synthesized by *Saccharomyces* yeast strains are valuable bioactive principles frequently used in medicine, food and cosmetic industry.

According to the given study, the content of β-glucanes in the biomass of *Saccharomyces cerevisiae* CNMN-Y-18 was 17.41% (nutritive mediumYPD) and 13.5% of dry cell weight (nutritive medium Rieder) (Table 2). For *Saccharomyces cerevisiae* CNMN-Y-19 content of β-glucanes vary in the limits of 16.23% … 16.70% of dry cell weight.

For both yeast strains significant values of mannoproteins were established. Specific for *Saccharomyces cerevisiae* CNMN-Y-18 was the amounts of mannoproteins that vary in limits 10.93…11.60% of dry cell weight (Table 2). Recent literature data indicated the amounts of mannoproteins up to 10.43% of dry biomass for some *Saccharomyces* yeast strains [24].

DISCUSSIONS

This paper contain data on Saccharomyces cerevisiae, selected as mannoprotein producers.

It is known fact, that the cell wall of the yeasts determines the form of the cell and the integrity of the microorganism during the growth and cellular division. Base structural components of the cell wall present main groups of polysaccharides: glucose polymers (β-glucanes, 47-60%), mannose polymers (mannanes, 30-40%) and polymers of N-acetylglucosamine (chitine, 0.6-3%), that together represents in average 90% of dry substance of the cell wall beside the presence to some quantities of lipids, enzymes, etc. [29].

Mannanes are associated covalently with proteins, forming glycoproteines, especially mannoproteins (5-20% proteins and 80-90% mannose) with the molecular weight between 20 and 200 kDa . Located preponderant in the outer layer of the cell wall, mannoproteins can form associations with β-1.3 and β-1.6 glucanes (by phosphodiester bonds) [17]. The important role of mannoproteins is the one of protection, assuring the rigidity the cell and maintaining the form this one in compliance with the stage of the cell cycle. These substances indicate properties of regulation cell wall permeability [19].

Researches on the structure of the cell wall and changes in biochemical composition, depending on changes in the environment are incomplete. Elucidation of the regulations related to metabolism can lead to the improving of various biotechnologies for production of microbial metabolites. The purpose of this study was to elucidate the phenotypic characters and biochemical composition of yeast strains selected as active producers of mannoproteins [29], the research necessary to supplement the information currently available.

The results of the investigations on phenotipical characters of the *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-19 offer advantages for the experimental study. The data accumulation for every yeast strain allows the insurance of the fermentative processes, this way improving the efficiency of the technologies of production of bioactive compounds how it would be in our case, the production of mannoproteins.

The realisation of the comparative studies of the composition of cell wall of *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-19 confirm the results described earlier. The establishment of the important quantities of polysaccharides at *Saccharomyces* yeasts confirm the perspective of its utilisation in the quality of biotehnological objects especially like the active producers of glucanes and mannoproteins. The dates obtained present teoretical and practical value for directed synthesis of valuable biologically active substances.

The phenotypical signs specific to *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-19 are important in the standard description of the species proposed as biotehnologic objects for industrial appointment. Chemical composition of *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-19 vary in broad limits and is characterized by a balanced protein (28.76...36.53%), carbohydrates (30.17...35.5%) and lipids content (6.2...6.6%) of dry biomass. The selected strains possess high antioxidant activity: catalase activity constitute 2035...2709 U/mg protein, superoxide dismutase activity - 153...240 U/mg protein. The polysaccharides complex constitute the base part of the cell wall of *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-19 yeast strains, characterized by the presence of 13.50...17.41% per dry cell weight of β-glucanes and 6.8...11.6% of mannoproteins. Thus, *Saccharomyces cerevisiae* CNMN-Y-18 yeast strain has been proposed to be included in list of microorganisms with high biotehnological potential for the production of mannoproteins.

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