



Effect of polyoxyethylene sorbitan monooleate (Tween 80) on exopolysaccharide and biomass composition of psychrophilic yeast

Snezhana RUSINOVA-VIDEVA^{1*} , Zhana PETKOVA² ,
Ginka ANTOVA² , Plamen STOYANOV^{3,4} ,
Tsvetelina MLADENOVA³ and Tzenka RADOUKOVA³

¹ *The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences,
139 Ruski Blvd, 4000 Plovdiv, Bulgaria*

² *Department of Chemical Technology, University of Plovdiv Paisii Hilendarski,
24 Tz. Assen, 4000 Plovdiv, Bulgaria*

³ *Department of Botany, University of Plovdiv Paisii Hilendarski, 24 Tz. Assen,
4000 Plovdiv, Bulgaria*

⁴ *Department of Pharmacognosy and Pharmaceutical Chemistry,
Medical University of Plovdiv, 15A V. Aprilov, 4000, Plovdiv, Bulgaria*

* corresponding author <jrusinova@abv.bg>

Abstract: Studies on the surface-active agents added during the cultivation process proved their effect on the synthesized bioproducts. The biosynthesis and accumulation of exopolysaccharide and biomass by Antarctic producer *Cryptococcus laurentii* AL₆₅ were studied in submerged cultivation using different carbon sources in the culture medium added to a one-percent concentration (pentoses, hexoses and oligosaccharides). Sucrose was chosen as the most appropriate carbon source for the cell growth and extracellular polymer biosynthesis. Absorption of sucrose by a strain producer was monitored and the residual sugars were traced in the dynamics of the fermentation process. The effect of polyoxyethylene sorbitan monooleate (Tween 80) on the cell growth and the accumulation of the synthesized biopolymer were studied for the first time with the Antarctic producer. The optimal increase in the amount of accumulated exopolysaccharide was recorded at a concentration of 6% Tween 80 at 24 h from the start of fermentation. The surfactant treatment contributed to an increase in the total amount of lipids in the cell, to the increased



biosynthesis of unsaturated fatty acids compared to saturated ones, and to the increased cellular synthesis of valuable metabolites such as phosphatidylinositol, phosphatidylserine and phosphatidylethanolamine. The impact of Tween 80 on intracellular and extracellular metabolites is a valuable asset in a possible scale-up of the process in industrial settings.

Keywords: Antarctic, *Cryptococcus laurentii*, biomass, lipids.

Introduction

Natural niches for the growth of psychrophilic microorganisms are Antarctic ecosystems, Arctic and Alpine glaciers and deep-sea waters (Buzzini and Margesin 2014a). Their presence in such environments have been thoroughly investigated (Vishniac 1999; Diaz and Fell 2000; Buzzini and Vaughan-Martini 2005). Extreme habitats, such as Antarctica, offer an attractive source of new microorganisms that produce bioactive molecules (Buzzini and Margesin 2014b). Microbiological and biotechnological research on Antarctic soil and plant samples taken from the territory of the Bulgarian Base on Livingston Island is connected with the study of the biodiversity of yeasts in this area as well as with the study of the selected strains as active producers of biological substances, *i.e.*, liposoluble metabolites, synthesized in the cell molecules with antioxidant activity (Pavlova *et al.* 2009, 2011; Dimitrova *et al.* 2010; Zlatanov *et al.* 2010).

In view of potential commercial applications and the growing tendency towards replacement of food, pharmaceutical and cosmetic ingredients with components of natural origin, the research in this field is expanding. Exopolysaccharides (EPSs) have been extensively used in food, pharmaceutical and cosmetic products (Freitas *et al.* 2011). Liposoluble substances which the biomass of microorganisms contains are usually rich in antioxidants and can be included in the composition of combined food and pharmaceutical additives (Dimitrova *et al.* 2010). Microbial lipids have practical application in the pharmaceutical, brewing and animal food production. Yeast strains be engineered to synthesize novel lipids for producing value-added oils and fats for the food and biomedical industries (Jacob 1993; Zlatanov *et al.* 2010).

Biotechnological processes are often subjected to optimization or modification (Debnath *et al.* 2021). Addition of an inducer to the nutrient medium can stimulate the production of the target product. Polyoxyethylene sorbitan monooleate was found to reduce the surface tension of the medium. It was often used as an inducer in the biotechnological production of enzymes, bacteriocins, organic acids, *etc.* It was proved that Tween 80 protects the enzyme production from external factors. Under optimized fermentation conditions, Tween 80 was successfully added to the culture medium for obtaining laccase by

Pleurotus ostreatus (Velásques-Quintero *et al.* 2022). This method also stimulated bacteriocin production by *Leuconostoc mesenteroides* (Da Silva Tomoto *et al.* 2022). The enhancement of GABA synthesis by *L. brevis* was also attributed to T80 (Jia *et al.* 2022). This surfactant was proposed for intensifying the cell growth of *Lactobacillus plantarum* and yeast *Yarrowia lipolytica*, their lipid production and lipase activity (Choi *et al.* 2021; Louhasakul *et al.* 2022). In some cases, only the lipid profile was affected without any impact on yeast growth (Grubišić *et al.* 2021). Polyoxyethylene sorbitan monooleate has been studied with respect to its use as a nourishing agent and as the only carbon source added to the nutrient medium in submerged cultivation of *Pleurotus tuber-regium* (Zhang and Cheung 2011a). Significant growth increase of thermophilic bacteria, accompanied by better uptake of the carbon source and a slight decrease of pH was observed when the medium was supplemented with Tween 80 (Fakhreddine *et al.* 1998). It is considered that the presence of polyoxyethylene sorbitan monooleate prevents the integration of the mycelium and affects both, the fatty acid composition and the amount of the cell lipids (Zhang and Cheung 2011a). The amount of oleic acid and to a lesser extent, the amount of palmitic, palmitoleic and stearic acids increases (Zhang and Cheung 2011a). In the presence of polyoxyethylene sorbitan monooleate, the unsaturated acids in the cell increase in comparison with the saturated ones (Zhang and Cheung 2011a). Its stimulating effect can be applied to other fermentation processes for production of different metabolites, including bioactive exopolysaccharide (Zhang and Cheung 2011b) or pectolytic enzymes of *Aspergillus niger* (Nemec *et al.* 2002).

The interest in exploring the microbial sources of biologically active substances is increasing globally. The relevance of the topic is determined by finding natural biomolecules that could replace synthetic ones. The aim of this research was to study the biosynthetic potential of Antarctic strain *Cryptococcus laurentii* AL₆₅ and the effects of surfactant Tween 80 on its biomass and extracellular polysaccharide synthesis.

Methods

Microorganism. — The research was performed with the Antarctic yeast strain *C. laurentii* AL₆₅, from the Antarctic Yeast Collection of the Institute of Microbiology of the Bulgarian Academy of Sciences, which was used as a metal ion biosorbent in previous studies (Rusinova-Videva *et al.* 2020).

Media and growth conditions. — The fermentation medium contained (g L⁻¹): sucrose 40; (NH₄)₂SO₄ 2.5; KH₂PO₄ 1.0; MgSO₄·7H₂O 0.5; NaCl 0.1; CaCl₂·2H₂O 0.1; yeast extract 1.0. The pentoses, hexoses and oligosaccharides were added at 1% concentration. The surfactant polyoxyethylene sorbitan monooleate was added to the culture medium at 24, 48 and 72 h at final

concentrations of 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0% v/v. The medium was sterilized for 30 min at 112°C. The submerged cultivation was conducted by shaking at 220 rpm for 120 h at 22°C. The inoculum from psychrophilic strain (10%) was obtained on a rotary shaker at 220 rpm at 22°C for 48 h.

Analytical methods. — The amount of synthesized exopolysaccharide (EPS) and the biomass was determined by the weighting method (Rusinova-Videva *et al.* 2020). Shimadzu high performance liquid chromatograph, equipped with a LC-20AD pump, a refractometric detector RID-10A and a software program LCsolution version 1.24 SP1 (Shimadzu Corporation, Kyoto, Japan) were used for the chromatographic analysis of the content of sucrose, fructose, and glucose. A Shodex® type Sugar SP0810 column with Pb^{2+} was used as a cation exchanger (5 μm , 300 mm x 8.0 mm) and the security column was Shodex SP-G (8 μm , 6 x 50 mm). The mobile phase was deionized water at a flow rate of 0.5 mL/min and a column temperature of 85°C. The solutions were degassed before use. The separation was run under an isocratic elution mode, at a sample volume of 20 μL . The resulting peaks were identified by comparing their retention time with that of sugar standards, with sucrose being at 16.02 min, for glucose at 17.12 min and for fructose at 23.55 min. For the quantification, the respective peak areas were used.

Isolation of glyceride oil and lipid analysis. — Biomass was air-dried and the oil was extracted with hexane in a Soxhlet extractor for 8 h (ISO 659:2014). The solvent was partly removed on a rotary vacuum evaporator, the residue was transferred into pre-weighed glass vessels and the rest of the solvent was removed under a stream of nitrogen to a constant weight to determine the oil content (ISO 659:2014).

The fatty acid composition of the extracted oils was determined by gas chromatography (GC) after transmethylation of the respective sample with 2% H_2SO_4 in CH_3OH at 50°C (ISO 12966-2:2011). GC was performed on a HP 5890 gas chromatograph equipped with a 75 m x 0.18 mm capillary column Supelco FP-2560 and a flame ionization detector. The column temperature was programmed from 140°C (5 min), at 4°C/min to 240°C (3 min); injector and detector temperatures were kept at 250°C. Hydrogen was the carrier gas at a flow rate of 0.8 mL/min. Fatty acids were identified by comparison of their retention times with those of a standard mixture of fatty acids subjected to GC under identical experimental conditions (ISO 12966-1:2014). Fatty acid methyl esters (FAME) were purified by thin-layer chromatography (TLC) on 20 x 20 cm plates covered with 0.2 mm silica gel 60 G (Merck, Darmstadt, Germany) layer with mobile phase hexane: diethyl ether 97:3 (v/v). The standard mixture of fatty acid methyl esters were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

A part of the air-dried biomass was subjected to Folch extraction (Folch *et al.* 1957) in order to isolate the polar lipids. The phospholipid classes were isolated by a variety of two-dimensional TLC on 20 x 20 cm glass plates with 0.2 mm Silica gel 60 G layer impregnated with aqueous $(\text{NH}_4)_2\text{SO}_4$ (1g in 100 mL

water). The first step was a mixture of chloroform: methanol: ammonia, 65:25:5 (v/v/v) and in the second – with chloroform: acetone: methanol: acetic acid: water, 50:20:10:10:5 (v/v/v/v/v) (Schneider and Daum 2006). The individual phospholipids were detected and identified by spraying with specific reagents: Dragendorff test (detection of choline-containing phospholipids), Ninhydrin spray (for phospholipids with free amino groups) and Schiff's reagent (for inositol containing phospholipids). Additional identification was performed by comparing the respective R_f values with those of authentic commercial standards subjected to silica gel TLC under identical experimental conditions. The quantification was carried out spectrophotometrically against a standard curve by measuring the phosphorous content at 700 nm after scrapping the respective phospholipid spot and mineralization of the substance with a mixture of perchloric acid and sulphuric acid, 1:1 by volume (ISO 10540-1:2014).

Statistics. — The measurements were performed in triplicate ($n = 3$) and the results were depicted as mean value $\pm$ standard deviation (SD). Data were analyzed by one-way ANOVA using SPSS (IBM SPSS Statistics 19, IBM Corp., Armonk, NY). A post-hoc analysis was performed using Duncan test with a significance level of $p < 0.05$.

Results

Cryptococcus laurentii AL₆₅ showed preference to some of the pentoses and hexoses accumulating higher amounts of biomass. The pentose sugars xylose and arabinose (Fig. 1A) and the hexose sugars mannose and galactose (Fig. 1B) were well absorbed and showed cell growth rates in the range of 1.12–3.27 g L⁻¹ (Fig. 1). The extracellular accumulation of exopolysaccharide did not show any correlation with that of the biomass. Hexoses and oligosaccharides were better transformed into exopolysaccharides in comparison with pentoses (Fig. 1B, C). Galactose, glucose and mannose proved to be the most suitable for accumulation of exopolysaccharide with commensurate values for the first two carbon sources (2.53 g L⁻¹ and 2.55 g L⁻¹), and 3.01 g L⁻¹ for mannose (Fig. 1B). The accumulated EPS with sucrose as a carbon source demonstrated similar values and it was selected for further studies.

The effect of sucrose concentration (3, 4 and 5%) as a carbon source for the biosynthetic processes was analysed. At 3% sucrose in the culture medium, the amount of the synthesized biomass reached *ca.* 5 g L⁻¹ at 96 h of the fermentation process. Exopolysaccharide was synthesised in the largest amount (*ca.* 2.3 g L⁻¹) at 72 h of the cultivation. The pH of the medium varied. By the 24th h of fermentation there was a sharp drop from 5.3 to 2.4, slowly changing afterwards reaching values of *ca.* 2 (Fig. 2A).

In the 4% sucrose samples, the accumulated amount of biomass was over 6 g L⁻¹ at 96 h. The obtained extracellular polymer reached its optimal amount

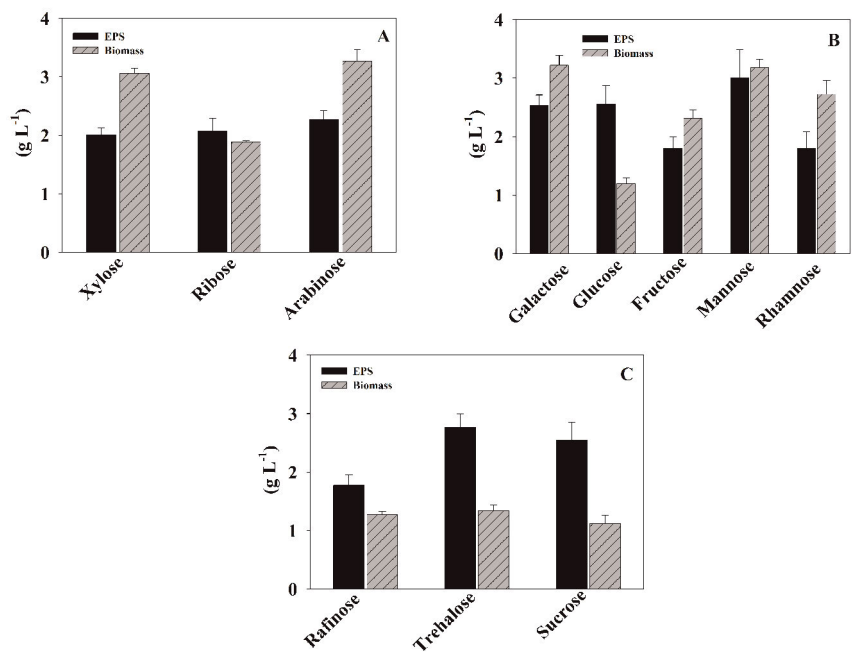


Fig. 1. Effect of pentoses (A), hexoses (B), and oligosaccharides (C) on cell growth and accumulation of exopolysaccharide (EPS) by *Cryptococcus laurentii* AL₆₅. Black shows exopolysaccharide (EPS) in g L⁻¹, grey shows biomass g L⁻¹.

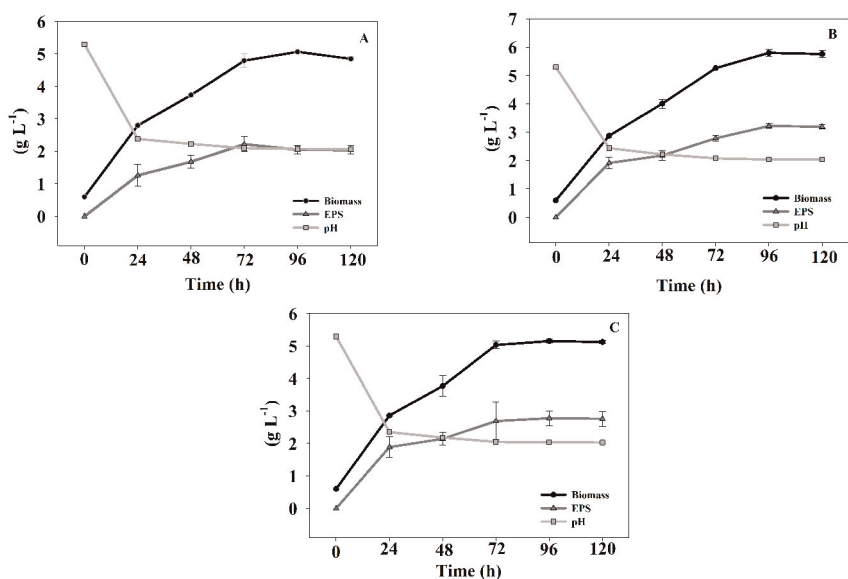


Fig. 2. Time course of biomass, exopolysaccharide (EPS) synthesis and pH, (A) 30 g L⁻¹ sucrose; (B) 40 g L⁻¹ sucrose; (C) 50 g L⁻¹ sucrose, at 22 C.

(ca. 3 g L⁻¹) at 96 h. The pH of the medium changes was similar to the pH of the 3% sucrose experiment (Fig. 2B). The reported cell growth in 5% sucrose medium was ca. 5.5 g L⁻¹ at 96 h. The synthesized biopolymer reached the highest values (ca. 2.8 g L⁻¹) at 96 h. There was no change in the pH curve in the dynamics of the process (Fig. 2C).

In the course of fermentation, the dynamics of sucrose utilization was established. By means of HPLC analysis, the products of sucrose hydrolysis, *i.e.*, glucose and fructose, were determined (Fig. 3). The reported amounts of sucrose decreased and, at 72th h, were below 10 g L⁻¹. After observation in the dynamics of the hydrolysis products, it was found that the glucose was consumed with preference over the fructose. At the end of the fermentation, the glucose was 4 g L⁻¹.

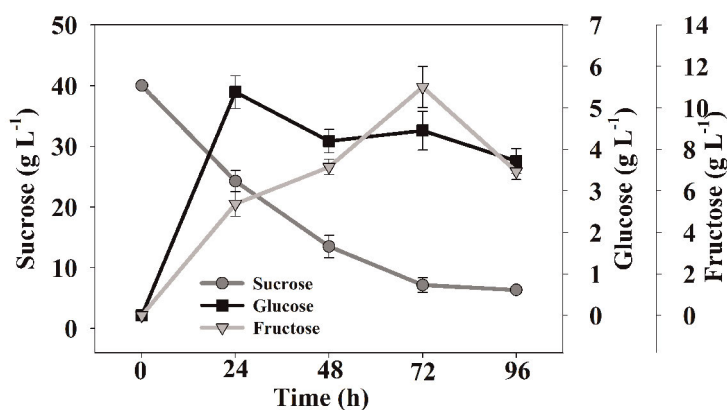


Fig. 3. The time course of sucrose hydrolysis, glucose, and fructose utilization during cultivation by the strain *Cryptococcus laurentii* AL₆₅.

Cryptococcus laurentii AL₆₅ has good biomass storage capacity and biopolymer synthesis. In submerged cultivation, it has a relatively short fermentation process in economically viable nutrient medium and natural protection against contamination.

The amount of synthesized biomass and extracellular biopolymer was studied in a modified medium in the dynamics of the fermentation process. In order to optimize the biosynthesis, the surfactant Tween 80 was added at concentrations of 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0% v/v. The influence of polyoxyethylene sorbitan monooleate was analyzed at 24, 48 and 72 h of fermentation.

The presence of the surfactant exerts a stimulating effect on the accumulation of exopolysaccharide in the real system. When added on the 24th hour, higher amounts of the biopolymer, compared to the control sample (96 h; Fig. 2B), were reported. A 30% increase and optimal results (ca. 4.5 g L⁻¹ EPS) were achieved with 6% polyoxyethylene sorbitan monooleate in the fermentation system (Fig. 4A).

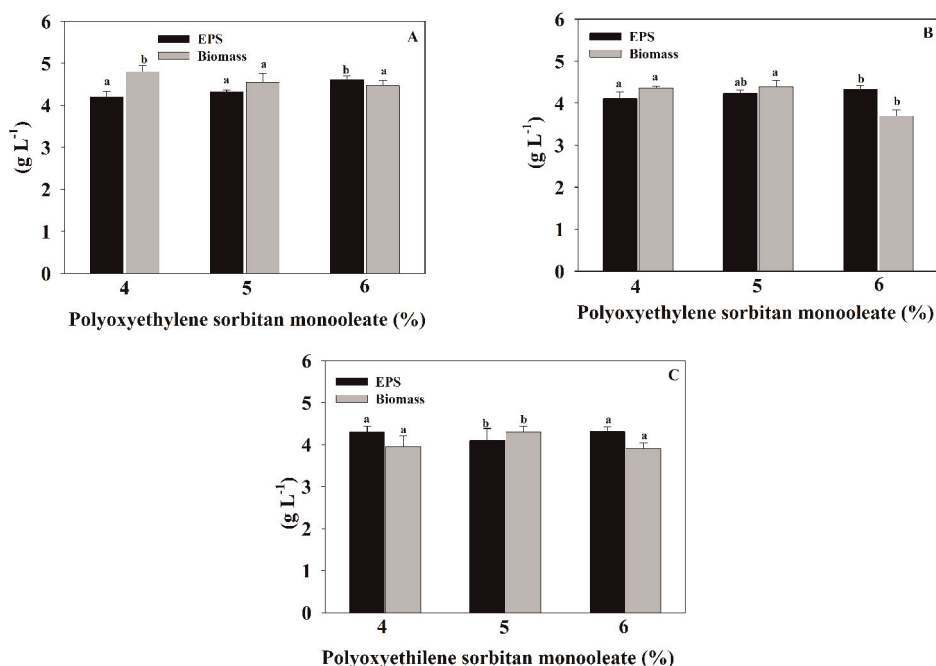


Fig. 4. Effect of polyoxyethylene sorbitan monooleate, on quantity of biomass and exopolysaccharide (EPS) by *Cryptococcus laurentii* AL₆₅, added at (A) 24th hours, (B) 48th hours, and (C) 72nd hours. Different letters mean significant differences ($p < 0.05$) of quantity of biomass and exopolysaccharide (EPS) at 4, 5 and 6% Tween 80.

From a biotechnological point of view no significant differences were observed in the results showing the accumulated extracellular polysaccharide after modification of the medium at 48 and 72 h (Fig. 4B, C). Compared with the control sample, slight inhibition of cell growth was observed when 6% surfactant was added at 48 and 72 h, as well as when 4% surfactant was added at 72 h (Fig. 4C). The results showing the effect of 0.1, 0.5, 1.0, 2.0, 3.0% surfactant on biosynthesis are not present due to minor response (S1).

The influence of the surfactant on the lipid content of the biomass was also studied. Samples obtained at 96 h, after treatment with Tween 80 at 24 h from the start of the fermentation process, were used. Table 1 shows the total lipid and phospholipid content in the lyophilized biomass of the strain in the presence of surfactant. The data were compared with that of the untreated biomass from our previous studies (Zlatanov *et al.* 2011).

The data on the fatty acid composition of the lipids from the Tween 80-treated and untreated biomass (Pavlova *et al.* 2012) were presented in Table 2. In the fatty acid composition of triacylglycerols, the unsaturated fatty acids (69.7%) were predominated, with the highest content being the oleic acid (57.0%). Of the saturated fatty acids, the palmitic (18.5%) and stearic acid (8.1%) were in the

Table 1.

Content of lipids and phospholipids in biomass of *C. laurentii* AL₆₅ with and without the surfactant. Different letters (lower-case a, b) in the same row mean significant differences ($p < 0.05$) while groups with the same letter are not significantly different.

Compound	Content with surfactant (%)	Content without surfactant after Zlatanov <i>et al.</i> (2011) (%)
Lipids	5.8±0.2 ^a	0.2±0.0 ^a
Phospholipids		
- in the lipids	45.2±1.7 ^a	50.0±0.5 ^b
- in the biomass	2.6±0.1 ^a	0.1±0.0 ^a

Table 2.

Fatty acid composition of lipids from biomass of *C. laurentii* AL₆₅ with and without addition of Tween 80. Different letters (lower-case a, b) in the same row mean significant differences ($p < 0.05$) while groups with the same letter are not significantly different.

Fatty acids		Content with surfactant (%)	Content without surfactant after Pavlova <i>et al.</i> (2012) (%)
C _{12:0}	Lauric acid	0.2±0.0 ^a	2.0±0.1 ^b
C _{12:1}	Lauroleic	not detected	0.3±0.0
C _{14:0}	Myristic acid	2.2±0.2 ^b	5.1±0.2 ^a
C _{14:1}	Myristoleic acid	0.2±0.0 ^a	0.2±0.0 ^a
C _{15:0}	<i>Pentadecanoic acid</i>	0.6±0.1 ^a	0.7±0.0 ^a
C _{16:0}	Palmitic acid	18.5±0.3 ^a	22.1±0.3 ^b
C _{16:1}	Palmitoleic acid	4.8±0.1 ^a	1.5±0.2 ^b
C _{17:0}	Margaric acid	0.5±0.0 ^a	0.3±0.0 ^b
C _{18:0}	Stearic acid	8.1±0.1 ^a	8.2±0.2 ^a
C _{18:1}	Oleic acid	57.0±0.4 ^a	48.2±0.6 ^b
C _{18:2}	Linoleic acid	5.6±0.2 ^a	9.1±0.2 ^b
C _{18:3}	Linolenic acid	-	0.4±0.0
C _{20:0}	Arachidic acid	-	0.6±0.0
C _{20:1}	Gadoleic acid	0.3±0.0 ^a	0.7±0.1 ^b
C _{20:2}	<i>Eicosadienoic acid</i>	1.8±0.1 ^b	0.3±0.0 ^a
C _{22:0}	<i>Behenic acid</i>	0.2±0.0 ^a	0.3±0.0 ^b
Saturated fatty acids		30.3	39.3
Unsaturated fatty acids		69.7	60.7

largest amounts. The amounts of linoleic acid and palmitoleic acid were very similar: 5.6 and 4.8% respectively. The remaining fatty acids were presented in relatively low values (from 0.2 to 2.2%).

Figure 5 illustrates the changes in the fatty acid composition of untreated and surfactant-treated biomass. Table 3 shows the individual phospholipid composition of the untreated and surfactant treated *C. laurentii* AL₆₅ biomass. In the surfactant-treated biomass, phosphatidic acids were predominated, followed by phosphatidylcholine, phosphatidylinositol and phosphatidylserine. The amount of other phospholipids were between 2.2 and 10.3%.

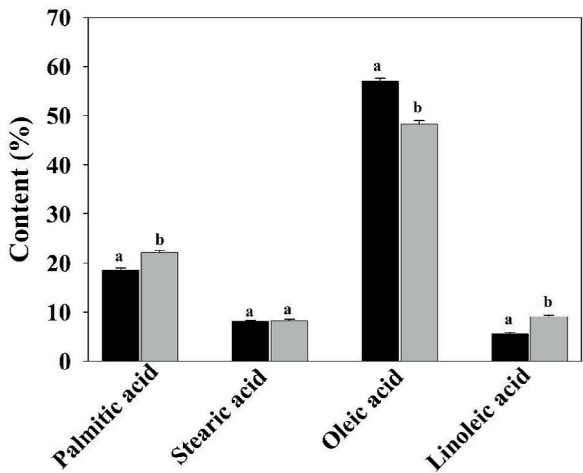


Fig. 5. The comparison of fatty acid composition of biomass received at cultivation with and without the surfactant; black - Control (Pavlova *et al.* 2012), gray - Tween 80. Different letters (lower-case) depict significant differences in the content of the fatty acids ($p < 0.05$). Groups with the same letter are not significantly different.

Table 3.

Individual phospholipid composition of *C. laurentii* AL₆₅ with and without the surfactant. Different letters (lower-case a, b) in the same row mean significant differences ($p < 0.05$) while groups with the same letter are not significantly different.

Phospholipids	Content with surfactant (%)	Content without surfactant after Zlatanov <i>et al.</i> (2011) (%)
Phosphatidylinositol	15.9±0.4	8.4±0.2 ^b
Phosphatidylserine	11.3±0.3	-
Phosphatidylcholine	18.4±0.5 ^a	13.7±0.2 ^b
Phosphatidylethanolamine	10.3±0.2 ^a	8.3±0.1 ^b

Table 3 - *continued*.

Phospholipids	Content with surfactant (%)	Content without surfactant after Zlatanov <i>et al.</i> (2011) (%)
Monophosphatidylglycerol	3.4±0.1 ^a	17.5±0.4 ^b
Diphosphatidylglycerol	2.2±0.1	-
Sphingomyelin	not detected	10.1±0.3
Phosphatidic acid	38.5±0.6 ^a	10.1±0.1 ^b
Lysophosphatidylcholine	-	13.6±0.4
Lysophosphatidylethanolamine	-	18.3±0.2

Discussion

The carbon source is the major component in the study of the composition of the fermentation media as it is the energy source for the culture. Different carbon sources were included in the culture medium for determination of the biosynthetic characteristics of the producer. As the most economically viable carbon source, sucrose is the most commonly used in industry.

Cryptococcus laurentii AL₆₅ has a good ability to biotransform sucrose into accumulated biomass and biopolymer in submerged cultivation. As a result of the conducted studies, the optimal amount of carbon source for accumulation of biomass in the fermentation process was found to be 4% sucrose. The optimal amount of biomass was *ca.* 6 g L⁻¹ and the shortening of the fermentation process duration from 120 h to 96 h makes it economically viable. After the 24th hour of fermentation, the natural course of pH decreased from 5.3 to 2.1. This is an advantage of the process due to the natural protection against contamination. As a primary metabolite, the biomass showed a definite course of growth phases, characteristic of microorganisms (Fig. 2A–C). In the dynamics of the bioprocess, the presence of exopolysaccharide was reported which gives additional information about the biosynthetic capabilities of the strain producer and provides grounds for a further research.

The described results are similar to the synthesized biomass of Antarctic yeast of the same genus (Pavlova *et al.* 2009; 2011; Rusinova-Videva *et al.* 2011). The optimal biomass synthesis of *C. laurentii* AL₁₀₀ was again at 96 h: 6.4 g L⁻¹ with 40 g L⁻¹ sucrose in the fermentation medium (Pavlova *et al.* 2011) and of *C. flavius* was at 120 h: 5.5 g L⁻¹ (Pavlova *et al.* 2009). Submerged culture studies of *C. laurentii* AL₆₂, selected as exopolysaccharide producer, showed 6.6 g L⁻¹ biomass accumulation at 72 h, but with a higher amount of carbon source (Rusinova-Videva *et al.* 2011).

The effect of the surfactant on the amount of accumulated biomass was negligible (Fig. 4). It was similar for the yeast *Trichosporon oleaginosus*, where only lipid accumulation was affected (Grubišić *et al.* 2021). For some bacteria, Tween 80 affects biomass accumulation proportionally (Taoka *et al.* 2011). There are studies on the use of polyoxyethylene sorbitan monooleate as an inducer in the culture medium. Zhang and Cheung (2011a) indicate that the addition of 0.3% of the surfactant on the fifth day of the fermentation increases the biomass by 51.3% and the exopolysaccharide by 41.8%. Tween 80 is also successfully used to induce some extracellular metabolites such as exopolysaccharides and enzymes in bacteria and fungi (Galindo and Salcedo 1996; Velásques-Quintero *et al.* 2022). Compared to other surfactants, polyoxyethylene sorbitan monooleate demonstrates better results in the increase of exopolysaccharide production (Hsieh *et al.* 2008).

In addition to our studies on the monosaccharide composition of the synthesized exopolysaccharide, we proved the presence of heteropolymer with the following composition: arabinose 34.9%, mannose 34.9%, glucose 16.7%, galactose 10.4%. It showed good emulsifying and stabilizing properties in oil/water system at a concentration of 2.5%, *i.e.*, the formed stable emulsion after centrifugation was 70% with no separated oil (Kuncheva *et al.* 2010). These properties of EPS determine its numerous practical applications. One aspect of the influence of Tween 80 on cell membranes is its ability to change their permeability (Grubišić *et al.* 2021). An increase in oleic acid is considered to be related to a proportional increase in the permeability of the cell membrane and hence the possibility to enhance the production of EPS (Zhang and Cheung 2011a). The control sample showed a lipid content of 0.2% with a phospholipid content of 50.0% in the lipid and 0.1% in the biomass (Zlatanov *et al.* 2011). Surfactant-treated biomass showed significantly higher oil content, as well as, synthesis of more phospholipids in the biomass compared to the control.

The results showed that oleic acid and, to a lesser extent, stearic acid had an increased accumulation of biomass compared to the control (Pavlova *et al.* 2012) (Fig. 5). On the other hand, the amount of palmitic and linoleic acids was decreasing. The same tendency was observed in *P. tuber-regium*, with the values of linoleic acids being significantly higher (Zhang and Cheung 2011a).

When compared with our previous studies (Zlatanov *et al.* 2011), significant differences were observed in both the qualitative and quantitative composition of the phospholipids in the treated and untreated yeast strain (Table 3). No presence of sphingomyelin, lysophosphatidylcholine and lysophosphatidylethanolamine in the surfactant treated biomass was found. In untreated biomass their values are 10.1, 13.6 and 18.3%, respectively. Lysophosphatidylethanolamine and monophosphatidylglycerol were with the highest content in the control sample. The amount of the remaining phospholipids ranges from 8.3 to 13.7% and no presence of diphosphatidylglycerol and phosphatidylserine was established. The addition of Tween 80 to *C. laurentii* AL₆₅ could be used to produce targeted phospholipids or to increase their amount.

Conclusions

In summary, *C. laurentii* AL₆₅ has good cell growth and ability to transform sucrose into exopolysaccharide, with levels of over 6 g L⁻¹ of accumulated biomass and an optimum of 3 g L⁻¹ for the extracellular biopolymer. The optimal parameters of the amount and the time of addition of the surfactant have been determined. A concentration of 6% on the 24th hour from the beginning of the fermentation process leads to an increase of exopolysaccharide production compared to the control. This, along with the distinctive pH change in the fermentation medium, resulting in natural protection against contamination, can be beneficial in large-scale manufacturing. Treatment of the strain with polyoxyethylene sorbitan monooleate showed a significant increase in lipid and phospholipid biosynthesis as well as in the amount of oleic acid in the fatty acid profile of the modified biomass. The described process could be implemented in industry and the obtained intracellular and extracellular products could be applied in cosmetics and pharmaceuticals.

Acknowledgements. — Authors are thankful to Kostantza Pavlova who kindly supplied the Antarctic yeast strains. Two reviewers whose comments greatly helped to improve this manuscript are acknowledged.

References

- Buzzini P. and Vaughan-Martini A. 2005. Yeast biodiversity and biotechnology. In: Rosa C. and Péter G. (eds.) *Biodiversity and ecophysiology of yeasts*. Springer: 533–559. doi: 10.1007/3-540-30985-3
- Buzzini P. and Margesin R. 2014a. Cold-adapted yeasts in Arctic habitats. In: Buzzini P. and Margesin R. (eds.) *Cold-adapted yeasts. Biodiversity, adaptation strategies and biotechnological significance*. Springer, Heidelberg New York Dordrecht London: 49–171.
- Buzzini P. and Margesin R. 2014b. Cold-adapted yeasts: a lesson from the cold and a challenge for the XXI century. In: Buzzini P. and Margesin R. (eds.) *Cold-adapted yeasts. Biodiversity, adaptation strategies and biotechnological significance*. Springer, Heidelberg New York Dordrecht London: 3–22.
- Choi G-H., Lee N-K. and Paik H-D. 2021. Optimization of medium composition for biomass production of *Lactobacillus plantarum* 200655 using response surface methodology. *Journal of Microbiology and Biotechnology* 31: 717–725. doi: 10.4014/jmb.2103.03018
- Da Silva Tomoto L., De Assis T.M., Filho F.C., Silva Araujo T.M., Vilver R. M., Santoyo M.C. and Gomes S.D. 2022. Production of bacteriocins by *Leuconostoc mesenteroides* using wastewater from the cassava starch industry as a growing medium. *Industrial Biotechnology* 18: 197–204. doi: 10.1089/ind.2021.0034
- Debnath A., Das B., Devi M. and Ram R. 2021. Fungal exopolysaccharides: types, production and application. In: Vaishnav A. and Choudhary D. (eds.) *Microbial polymers, applications and ecological perspectives*. Springer Nature, Singapore: 45–68.
- Diaz M. and Fell J. 2000. Molecular analyses of the IGS & ITS regions of rDNA of the psychrophilic yeasts in the genus *Mrakia*. *Antonie van Leeuwenhoek* 77: 7–12. doi: 10.1023/A:1002048008295

- Dimitrova S., Pavlova K., Lukanov L. and Zagorchev P. 2010. Synthesis of coenzyme Q10 and β -caroten by yeasts isolated from Antarctic soil and lichen in response to ultraviolet and visible radiations. *Applied Biochemistry and Biotechnology* 162: 795–804. doi: 10.1007/s12010-009-8845-z
- Fakhreddine L., Kademi A., Ait-Abdelkader N. and Baratti J. 1998. Microbial growth and lipolytic activities of moderate thermophilic bacterial strain. *Biotechnology Letters* 20: 879–883. doi: 10.1023/A:1005371727699
- Folch J., Lees M. and Sloane–Stanley G. H. 1957. A simple method for isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226: 498–509.
- Freitas F., Alves V. and Reis M. 2011. Advances in bacterial exopolysaccharides: from production to biotechnological applications. *Trends in Biotechnology* 29: 388–398. doi: 10.1016/j.tibtech.2011.03.008
- Galindo E. and Salcedo G. 1996. Detergents improve xanthan yield and polymer quality in cultures of *Xanthomonas campestris*. *Enzyme and Microbial Technology* 19: 145–149. doi: 10.1016/0141-0229(95)00215-4
- Grubišić M., Mihajlovski K., Gručić A., Beluhan S., Šantek B. and Šantek M. 2021. Strategies for improvements of lipid production by yeast *Trichosporon oleaginosus* from lignocellulosic biomass. *Journal of Fungi* 7: 934. doi: 10.3390/jof7110934
- Hsieh C., Wang H-L., Chen C-C., Hsu T-H. and Tseng M-H. 2008. Effect of plant oil and surfactant on the production of mycelia biomass and polysaccharides in submerged culture of *Grifola frondosa*. *Biochemical Engineering Journal* 38: 198–205. doi: 10.1016/j.bej.2007.07.001
- ISO 10540-1:2014. Animal and vegetable fats and oils. Determination of phosphorus content. Part 1: Colorimetric method.
- ISO 12966-2:2011. Animal and vegetable fats and oils. Gas chromatography of fatty acid methyl esters. Part 2: Preparation of methyl esters of fatty acids.
- ISO 12966-1:2014. Animal and vegetable fats and oils. Gas chromatography of fatty acid methyl esters. Part 1: Guidelines on modern gas chromatography of fatty acid methyl esters.
- ISO 659:2014. Oilseeds. Determination of oil content (Reference method).
- Jacob Z. 1993. Yeast lipid biotechnology. In: Neidelman S. and Laskin A. (eds.) *Advances in applied microbiology*. Academic Press, Inc., California: 185–212.
- Jia M., Zhu Y., Wang L., Sun T., Pan H. and Li H. 2022. pH auto-sustain-based fermentation supports efficient gamma-aminobutyric acid production by *Lactobacillus brevis* CD0817. *Fermentation* 8: 208. doi: 10.3390/fermentation8050208
- Kuncheva M., Panchev I., Pavlova K., Rusinova-Videva S. and Georgieva K. 2010. Emulsifying and rheological properties of exopolysaccharides synthesised by psychrophilic yeast. *Scientific works LVII* 2: 113–117.
- Louhasakul Y. and Cheirsilp B. 2022. Potential use of industrial by-products as promising feedstock for microbial lipid and lipase production and direct transesterification of wet yeast into biodiesel by lipase and acid catalysis. *Bioresource Technology* 348: 126742. doi: 10.1016/j.biortech.2022.126742
- Nemec T. and Jernejc K. 2002. Influence of Tween 80 on lipid metabolism of an *Aspergillus niger* strain. *Applied Biochemistry and Biotechnology* 101: 229–238. doi: 10.1385/ABAB:101:3:229
- Pavlova K., Panchev I., Kratchanova M. and Gocheva M. 2009. Production of an exopolysaccharide by Antarctic yeast. *Folia Microbiologica* 54: 343–348. doi: 10.1007/s12223-009-0049-y
- Pavlova K., Rusinova-Videva S., Kuncheva M., Kratchanova M., Gocheva M. and Dimitrova S. 2011. Synthesis and characterization of an exopolysaccharide from Antarctic yeast strain *Cryptococcus laurentii* AL₁₀₀. *Applied Biochemistry and Biotechnology* 163: 1038–1052. doi: 10.1007/s12010-010-9107-9
- Pavlova K., Zlatanov M., Antova G., Angelova-Romova M. and Georgieva K. 2012. Biosynthesis and characterization of exopolysaccharides and lipids from Antarctic yeasts. *Biotechnology and Biotechnological Equipment* 24: 3123–3128. doi: 10.5504/BBEQ.2012.0038

- Rusinova-Videva S., Pavlova K. and Georgieva K. 2011. Effect of different carbon sources on biosynthesis of exopolysaccharide from Antarctic strain *Cryptococcus laurentii* AL₆₂. *Biotechnology and Biotechnological Equipment* 25: 80–84. doi: 10.5504/BBEQ.2011.0121
- Rusinova-Videva S., Nachkova S., Adamov A. and Dimitrova-Dyulgerova I. 2020. Antarctic yeast *Cryptococcus laurentii* (AL₆₅): biomass and exopolysaccharide production and biosorption of metals. *Journal of Chemical Technology and Biotechnology* 95: 1372–1379. doi: 10.1002/jctb.6321
- Schneider R. and Daum G. 2006. Analysis of yeast lipids. In: Xiao W. (ed) *Yeast protocol, 2nd ed. Methods in Molecular Biology*. Humana Press Inc., Totowa, New Jersey: 75–84.
- Taoka Y., Nagano N., Okita Y., Izumida H., Sugimoto S. and Hayashi M. 2011. Effect of Tween 80 on the growth, lipid accumulation and fatty acid composition of *Thraustochytrium aureum* ATCC34304. *Journal of Bioscience and Bioengineering* 111: 420–424. doi: 10.1016/j.jbiosc.2010.12.010
- Vishniac H. S. 1999. Psychrophilic yeasts. In: Seckbach J. (ed) *Enigmatic microorganisms and life in extreme environments*. Academic Publishers, The Netherlands: 315–321. doi: 10.1007/978-94-011-4838-2
- Velásques-Quintero C., Merino-Restrepo A. and Hormaza-Anaguano A. 2022. Production, extraction, and quantification of laccase obtained from an optimized solid-state fermentation of corn cob with white-rot fungi. *Journal of Cleaner Production* 370: 133598. doi: 10.1016/j.jclepro.2022.133598
- Zhang B. and Cheung P. 2011a. A mechanistic study of the enhancing effect of Tween 80 on the mycelia growth and exopolysaccharide production by *Pleurotus tuber-regium*. *Bioresource Technology* 102: 8323–8326. doi: 10.1016/j.biortech.2011.06.021
- Zhang B. and Cheung P. 2011b. Use of stimulatory agents to enhance the production of bioactive exopolysaccharide from *Pleurotus tuber-regium* by submerged fermentation. *Journal of Agriculture Food Chemistry* 59: 1210–1216. doi: doi.org/10.1021/jf104425w
- Zlatanov M., Pavlova K., Antova G., Angelova-Romova., Georgieva K. and Rusinova–Videva S. 2010. Biomass production by Antarctic yeast strains: an investigation on the lipid composition. *Biotechnology and Biotechnological Equipment* 24: 2096–2101. doi: 10.2478/V10133-010-0084-5
- Zlatanov M., Pavlova K., Angelova-Romova M., Antova G., Georgieva K. and Rusinova-Videva S. 2011. Phospholipid composition in biomass of Antarctic yeast strains. *Scientific researches of the Union of Scientists in Bulgaria-Plovdiv* 13: 116–120.

Received 10 September 2022

Accepted 26 January 2023