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The exopolysaccharides biosynthesis by *Candida* yeast depends on carbon sources

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ABSTRACT

Background: The exopolysaccharides (EPS) produced by yeast exhibit physico-chemical and rheological properties, which are useful in the production of food and in the cosmetic and pharmaceutical industries as well. The effect was investigated of selected carbon sources on the biosynthesis of EPS by *Candida famata* and *Candida guilliermondii* strains originally isolated from kefirs.

Results: The biomass yields were dependent on carbon source (sucrose, maltose, lactose, glycerol, sorbitol) and ranged from 4.13 to 7.15 g/L. The highest biomass yield was reported for *C. guilliermondii* after cultivation on maltose. The maximum specific productivity of EPS during cultivation on maltose was 0.505 and 0.321 for *C. guilliermondii* and *C. famata*, respectively. The highest EPS yield was found for *C. guilliermondii* strain. The EPS produced under these conditions contained 65.4% and 61.5% carbohydrates, respectively. The specific growth rate (μ) of *C. famata* in medium containing EPS as a sole carbon source was 0.0068 h^{-1} and 0.0138 h^{-1} for *C. guilliermondii* strain.

Conclusions: The most preferred carbon source in the synthesis of EPS for both *Candida* strains was maltose, wherein *C. guilliermondii* strain showed the higher yield of EPS biosynthesis. The carbon source affected the chemical composition of the resulting EPS and the contribution of carbohydrate in the precipitated preparation of polymers was higher during supplementation of maltose as compared to sucrose. It was also found that the EPS can be a source of carbon for the producing strains.

1. Introduction

Extracellular polysaccharides (EPS) are produced by yeast species from the *Candida*, *Cryptococcus*, *Lipomyces*, *Pichia*, *Rhodotorula*, and *Sporobolomyces* genera [1,2,3,4]. Some of these polysaccharides exhibit specific physico-chemical and rheological properties, which are useful in the production of food and in the cosmetic and pharmaceutical industries as well [5]. EPS containing more than 50% mannose in their composition are characterized by biological activity, *e.g.* linear mannans from *Rhodotorula mucilaginosa*, which exhibit anti-tumor activity in experimental animals [6], or glucomannan from *Candida utilis,* which possesses antioxidant activity [4]. Polymers from *Rhodotorula glutinis* exhibit antioxidant, antiviral and antitumor activity [7] and mannans produced by *R. glutinis* AHU 3479 have proved to serve as an immunoreactive antigen in serological diagnosis of leptospirosis [8].

So far, the only commercially produced extracellular fungal polymer is pullulan. It is used as a thickener and as a component of edible coatings [9]. Also, exopolysaccharides produced by other yeasts could constitute an alternative to similar but chemically synthesized ones. EPS are renewable raw materials and satisfy the environmental protection standards for classification as bioproducts. For their industrial production, efficient producer yeast strains, knowledge of the regulation of biosynthetic pathways, optimization of cultivation conditions and efficient methods for purifying the product are necessary.

EPS producers are sought mainly among cryophilic microorganisms inhabiting soil and in cold Antarctic ecosystems. Efficient strains of Cryptococcus, Rhodotorula [10] and Sporobolomyces [11,12] genera have been isolated. However, little is known about the biosynthesis of exopolysaccharides by yeast from the food environment.

The production of exopolysaccharides is associated with the secondary metabolism of yeast, and their structure as well as physico-chemical properties depend on many factors. Culture medium composition, in particular the source of carbon and nitrogen, and culture conditions, the degree of oxygenation and temperature [11,13, 14], have the greatest impact on the amount and characteristics of polymers produced during fermentation. Most strains studied so far produced EPS in media containing such carbon sources as pentoses, hexoses, disaccharides and triose. For instance, strains from

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Sporobolomyces and *Cryptococcus* genera produced various amounts of EPS in mineral media containing, among others, glucose, sucrose, xylose, ribose, arabinose, galactose, mannose, rhamnose, raffinose, and trehalose [2,15].

In our earlier study, we isolated *Candida* yeast strains from Polish kefirs [16]. Most of the strains were able to synthetize extracellular polysaccharides during incubation in mineral medium with sucrose. *Candida famata* and *Candida guilliermondii* strains were selected as the best EPS producers [17].

The yeast *C. famata* is a member of the group of so-called "flavinogenic yeasts" which overproduce riboflavin under conditions of iron limitation. A modified strain accumulated up to 16.4 g/L of riboflavin in optimized medium in a laboratory bioreactor during fed-batch fermentation [18]. Some *C. famata* strains overproduce flavin adenine dinucleotide (FAD) [19]. *C. famata* is known to demonstrate glucoamylase activity [20]. The data obtained indicate the probiotic potential of this strain [21]. For example, *C. famata* Y5 strains have been studied for their cytotoxicity, adhesion, surface properties, and hemolytic activity, and their survival in simulated gastric and intestine environments. The yeast *C. guilliermondii* FTI 20037 is well-known for its ability to produce xylitol from xylose [22].

It can utilize and ferment high concentrations of each of the hexoses commonly found in lignocellulosic hydrolysates. The hydrolysate was converted to xylitol with a yield of 0.48 g/g (53% of the theoretical maximum) [23]. Little is known about the biosynthesis of EPS by yeast from the *Candida* genus. *C. utilis* ATCC 42402 produces extracellular glucomannan in the amount of 1.68 g/L. The crude EPS contained 98% carbohydrates which consisted of glucose and mannose in the molar ratio 5.7:1 [24].

The objective of the study was to investigate the influence of carbon sources on exopolysaccharide biosynthesis by *C. famata* and *C. guilliermondii* strains isolated from kefirs.

2. Materials and methods

2.1. Strain and culture conditions

Biological material consisted of two yeast strains derived from the Museum of Pure Cultures, Department of Biotechnology and Microbiology of Food WULS-SGGW: *C. famata*, *C. guilliermondii*, isolated from Polish kefirs [16]. The strains were stored in a refrigerator in YPD medium (composed of 2% glucose, 2% peptone, 1% yeast extract, pH 5.6) at a passage frequency every 3–4 weeks.

2.2. Culture conditions

To study the biosynthesis of EPS, mineral medium (pH 5.6) was composed of: 0.2% of $(NH_4)_2SO_4$; 0.1% of KH_2PO_4; 0.05% of $MgSO_4 \times 7H_2O$; 0.01% of $CaCl_2$; 0.01% of NaCl and 0.1% yeast extract. This medium was supplemented with an appropriate carbon source to a concentration of 5% after sterilization. Sucrose, lactose, maltose, glycerol and sorbitol were used as the carbon source. The characteristics of the respective media are shown in Table 1. All media were sterilized by autoclaving at 121°C for 20 min. Carbon source solutions were sterilized by autoclaving at 117°C for 20 min. In order to obtain inoculum, biological material was transferred from slants to 100 cm^3 of YPD medium with the use of an inoculation loop followed by 48 h of culturing on a reciprocating shaker (200 rpm) at 28°C. From such prepared culture, 5 cm^3 was collected to inoculate 100 cm^3 of mineral medium. The cultures assigned to EPS production were carried out under aerobic conditions on a reciprocating shaker (200 rpm) at 28°C for 96 h. To investigate the effect of incubation time on the biosynthesis of EPS, after selection of the most preferred carbon source, cultivating was extended to 120 h.

Table 1
Characteristics of the tested media depending on carbon source.

Carbon source	Carbon source concentration %	Formula of carbon source	Molar mass of carbon source g/mol	Molar conc. of carbon source mol/l	C:N:P
Sucrose	5	$C_{12}H_{22}O_{11}$	342.30	0.146	49.6:1:0.54
Lactose	5	$C_{12}H_{22}O_{11}$	342.30	0.146	49.6:1:0.54
Maltose	5	$C_{12}H_{22}O_{11}$	342.30	0.146	49.6:1:0.54
Sorbitol	5	$C_6H_{14}O_6$	182.17	0.274	46.6:1:0.54
Glycerol	5	$C_3H_8O_3$	92.09	0.543	46.1:1:0.54

2.3. Determination of biomass

In order to determine the biomass yield after completion of incubation, post-culture fluid was transferred to a dried and weighed extraction thimble and centrifuged at 2000 × g, 20 min (Eppendorf Centrifuge 5804R). Supernatant was decanted and the biomass precipitate was washed twice with sterile distilled water, followed by drying to constant weight at 105°C (SML 32/250 Zelmed drier, Poland). The result of biomass yield was given in grams of dry substance per liter of medium (g/L).

2.4. Determination of optical density

Optical density (OD) of culture was determined after centrifugation of 2 cm^3 of culture at 1610 × g, 5 min (Eppendorf mini Spin plus). The supernatant was decanted, and the biomass precipitate was suspended in 2 cm^3 of distilled water. The OD value was measured spectrophotometrically at a wavelength of 600 nm against distilled water (UV — 1800 UV/VIS, RayLeigh Analytical Instrument).

2.5. Determination of exopolysaccharide content

The amount of EPS was determined according to Pavlova [25]. Total volume of 50 cm^3 of culture medium was transferred to extraction thimbles of known mass and centrifuged at 6000 × g for 30 min. Then, 15 cm^3 of supernatant was collected and 30 cm^3 of ethanol (analytical grade, 96%) was added. The samples were left at 4°C for 24 h to obtain EPS precipitate. The precipitate was centrifuged for 30 min at 6000 × g, washed with ethanol and re-centrifuged under identical conditions. Precipitated EPS was dried at 80°C for 2 h, and then weighed (RADWAG PS 750/X, Poland). The crude EPS amount was given as g/L. The pH value during cultivation was determined using a Conbest CP50 pH meter.

2.6. Determination of sugar content in medium and in exopolysaccharides

The content of maltose in medium, as reducing carbohydrates, was analyzed using the colorimetric method (λ = 540 nm) with 3,5-dinitrosalicylic acid [26]. The amount of total sugars in exopolysaccharides was determined after acid hydrolysis (72% of H_2SO_4) according to the methodology described by Bzducha-Wróbel et al. [27].

2.7. The kinetics parameter calculation

The kinetics parameters for average yields of exopolysaccharide ($Y_{p/s}$) efficiency of biomass ($Y_{x/s}$) and specific production ($Y_{p/x}$) were calculated by the equations:

$$\begin{aligned} Y_{p/x} &= P/x \\ Y_{p/s} &= P/(M0-M) \\ Y_{x/s} &= x/(M0-M) \end{aligned} \qquad \text{[Equation 1]}$$

where x: yield of dry biomass (g/L); P: maximum exopolysaccharide concentration (g/L); M_0: initial concentration of maltose (g/L); M: residual maltose concentration (g/L).

2.8. Culture conditions in mineral medium with crude EPS as a source of carbon

After culture in mineral medium with maltose (5%), the biomasses of both strains were sterile centrifuged at 2000 × g, 20 min, (Eppendorf centrifuge 5804R), washed twice and diluted in sterile saline to obtain 10^5 cells /cm^3 approximately. The cells of *C. guilliermondii* were taken after 72 h of culture, and the cells of *C. famata* at 96 h, which was addicted to the maximum content of EPS in the culture medium. The cell suspensions formed the inocula for cultivation in mineral medium containing crude EPS (1%) as the source of carbon. The crude EPS was precipitated from mineral medium containing maltose at the same time as the yeast cells were also precipitated (according to the previously described method). Growth on a substrate with a suitable EPS was performed using Honeycomb plates in Bioscreen C (Oy Growth Curves Ab. Ltd, Helsinki, Finland) and a flask on a reciprocating shaker. Control cultures were cultures on mineral medium without a carbon source. In addition, sterile mineral medium containing EPS as a carbon source was incubated. The aim was to investigate the changes in the density of the medium during incubation. Honeycomb plates were filled with 250 μL of medium, and inoculated with 25 μL of yeast cell suspension. Flasks were filled with 50 cm^3 of suitable medium and inoculated with 5 cm^3 of yeast cell suspension. Cultures were grown for 96 h at 28°C with medium agitation during incubation in Bioscreen C and 200 rpm on a shaker. According to the producer's recommendations, a broadband filter (λ = 420–580 nm) was selected for OD measurements. The OD of the culture was measured every 15 min. The number of yeast cells was assayed every 24 h during flask culture using the plate method.

The coefficient of specific growth rate (μ) in time (t) was calculated from the formula:

$$\mu(t) = (\ln OD_f - \ln OD_i)/(t_f - t_i) \quad \text{[Equation 2]}$$

where OD_f: final OD in the log phase; OD_i: initial OD in the log phase; t_f: time of log-phase termination; and t_i: time of log-phase onset.

2.9. Statistical analysis

Statistical analysis was performed using Statistica Ver 10. Multivariate analysis of variance was used in the analysis. Homogeneous groups were determined using Tukey test, at a significance level of $\alpha \leq 0.05$. The results were presented as averages from three independent experimental series.

3. Results and discussion

3.1. Influence of carbon sources on the growth and synthesis of exopolysaccharides

The *C. famata* strain exhibited growth in all experimental media, while the biomass yield and the optical density were independent of the carbon source (Table 2). The largest biomass yield for the *C. famata* strain (6.60 g/L) was found in medium with sorbitol. In the medium with sucrose and lactose, the same strain was characterized by the lowest content of biomass, which did not exceed 5 g/L. The *C. guilliermondii* strain showed no growth in medium with lactose as the only carbon source, which confirmed an inability to assimilate sugar [28,29]. The highest biomass yield of (7.15 g/L) for the *C. guilliermondii* strain was reported after 96 h of cultivation in medium containing maltose.

In the medium with sucrose, *C. famata* and *C. guilliermondii* strains produced similar amounts of EPS until 72 h, *i.e.* 1.65 g/L and 1.72 g/L, respectively. During the next 24 h of cultivation, significantly higher amounts (above 2 g/L) of polymers were found in the *C. guilliermondii* culture. In studies by other authors [2,14,15], sucrose was considered the most suitable carbon source for the production of EPS by producer yeast strains. In medium containing 4% sucrose, *Cryptococcus laurentii* AL_{62}, *Sporobolomyces salmonicolor* AL_1, and *Sporobolomyces salmicolor* AL_{36} strains produced more than 5 g EPS/L [2,10]. In contrast to sucrose, *C. utilis* ATCC 42402 produced extracellular glucomannans in media containing 5% glucose [24]. It should be emphasized that no other publications evaluating the impact of different carbon sources on the synthesis of EPS by non-pathogenic strains of the *Candida* genus have been published so far.

Table 2
The effect of carbon source and time of cultivation on optical density, pH changes, biomass yield and the exopolysaccharide production by *Candida* strains in mineral medium.

Source of carbon/ time of incubation		OD	pH	Biomass g/L	EPSg/L
C. famata					
Sucrose	72 h	2.77	$2.59^{a,b}$	$4.73 \pm 0.40^{a,b}$	1.65 ± 0.11^{d}
	96 h	2.92	$2.44^{a,b}$	4.13 ± 0.11^{a}	1.75 ± 0.12^{d}
Lactose	72 h	2.85	$2.35^{a,b}$	$4.20 \pm 0.21^{a,b}$	$0.72 \pm 0.11^{a,b}$
	96 h	2.85	$2.23^{a,b}$	$4.61 \pm 0.21^{a,b}$	1.04 ± 0.09^{b}
Maltose	72 h	3.00	$2.54^{a,b}$	$6.37 \pm 0.51^{a,b}$	$1.89 \pm 0.12^{d,e}$
	96 h	3.12	$2.33^{a,b}$	$6.54 \pm 0.19^{a,b}$	$2.10 \pm 0.23^{d,e}$
Sorbitol	72 h	2.76	$2.18^{a,b}$	$6.05 \pm 0.25^{a,b}$	0.42 ± 0.08^{a}
	96 h	2.95	$2.08^{a,b}$	$6.60 \pm 0.05^{a,b}$	0.99 ± 0.06^{b}
Glycerol	72 h	2.76	$2.29^{a,b}$	$4.69 \pm 0.25^{a,b}$	0.56 ± 0.07^{a}
	96 h	3.05	$2.11^{a,b}$	$5.43 \pm 0.24^{a,b}$	1.20 ± 0.02^{c}
C. guilliermondii					
Sucrose	72 h	2.86	$2.35^{a,b}$	$5.82 \pm 0.11^{a,b}$	1.72 ± 0.07^{d}
	96 h	2.94	$2.32^{a,b}$	7.00 ± 0.23^{b}	2.10 ± 0.02^{e}
Lactose	72 h	NA	NA	NA	NA
	96 h	NA	NA	NA	NA
Maltose	72 h	2.99	$2.28^{a,b}$	$5.90 \pm 0.21^{a,b}$	2.98 ± 0.39^{f}
	96 h	2.98	1.92^{a}	7.15 ± 0.19^{b}	1.74 ± 0.27^{d}
Sorbitol	72 h	2.64	2.78^{b}	$4.67 \pm 0.18^{a,b}$	$0.81 \pm 0.02^{a,b}$
	96 h	2.81	$2.22^{a,b}$	$5.29 \pm 0.18^{a,b}$	0.44 ± 0.08^{a}
Glycerol	72 h	2.85	$2.26^{a,b}$	6.94 ± 0.15^{b}	1.31 ± 0.02^{c}
	96 h	2.94	$2.18^{a,b}$	$6.27 \pm 0.22^{a,b}$	$0.76 \pm 0.11^{a,b}$

Values represent the mean ± SD from assays of three samples. The same letter indices indicate a lack of a significant difference between both strains results, presented in columns.

Maltose appeared to be the preferred carbon source for the synthesis of EPS by the tested Candida strains in the mineral medium (Table 1). After 96 h of cultivation of *C. famata* in medium containing this sugar, the maximum amount of EPS (2.10 g/L) was reported. Similarly, *C. guilliermondii* yeast produced the most exopolysaccharides during utilization of maltose as a carbon source. EPS content reached nearly 3 g/L, which represented about 73% more than the amount of EPS produced over the same time period in medium with sucrose. When maltose was used as the only carbon source, the cultivation time significantly affected the amount of exopolysaccharides depending on the strain. For the *C. guilliermondii* strain, the amount of EPS determined decreased almost by half on the fourth day of cultivation in relation to the third, while the content of exopolysaccharides produced by *C. famata* increased.

Lactose was not found to be the preferred carbon source for the synthesis of EPS by *C. famata*. The maximum amount of extracellular polysaccharides was slightly more than 1 g/L, and this was lower than the amount of EPS in medium with sucrose. The *C. guilliermondii* strain did not assimilate lactose; therefore, exopolysaccharide content in medium with this sugar was not determined.

The ability by Candida strains to produce EPS based on carbon sources other than non-carbohydrates has not been studied so far. In the present study, sorbitol and glycerol were used. Efficient yeast growth was observed in media supplemented with these alcohols, as biomass yields of both strains did not differ significantly from those obtained in media with disaccharides (Table 2). In the presence of sorbitol, yeasts produced not more than 0.99 g EPS/L, and approximately 1.31 g EPS/L in the presence of glycerol (Fig. 1). The amount of exopolysaccharides was significantly lower than in medium

with sucrose; therefore, sorbitol and glycerol should be considered unfavorable carbon sources in the synthesis of EPS.

3.2. Influence of C:N ratio on the synthesis of EPS

Apart from the influence of carbon source, an important role in the biosynthesis of EPS is played by the source and the concentration of nitrogen. Ammonium sulfate is considered a preferred source of N, and a 0.2% concentration in the medium is its optimal dose [13,14]. Some literature data indicate [13] that yeasts from the Rhodotorula genus are able to produce the most exopolysaccharides when the C:N ratio in the medium is 15:1. These authors suggested that a higher C:N ratio causes activation of the accumulation mechanism of other cell storage substances, including fats [30]. It should be noted that C:N ratios in mineral media with sucrose, maltose or lactose were identical (C:N 49,6:1) in terms of the elemental composition of these saccharides (Table 1). Nevertheless, the amount of produced EPS varies significantly depending on the source of carbon. Therefore, the C:N ratio could not have a direct effect on the biosynthesis of EPS. Yeasts of the Candida genus produce hydrolytic enzymes, maltase and sucrase [31]. The lack of any significant differences in the biomass yield and optical density of tested strains (Table 2) may evidence that the rate of sugar assimilation (determined by the activity of hydrolytic enzymes) does not affect the synthesis of EPS.

Biosynthesis of EPS was dependent on the strain, carbon source, and also on the cultivation time. *C. famata* cells produced significantly more EPS after 96 h than after 72 h, irrespective of the carbon source tested. However, during the cultivation of *C. guilliermondii,* in media containing maltose, glycerol and sorbitol, the amount of exopolymers determined on the fourth day in relation to 72 h decreased. More EPS were found on the fourth d, however, in the case of the use of sucrose as a carbon source.

3.3. Phenomenon of acidification during EPS biosynthesis

In each post-culture medium, a significant increase in the active acidity of the culture environment was reported. After 3 d, pH of the media ranged between 2.18 and 2.78, depending on the strain and the carbon source, but on the fourth day variation was not significant. The lowest pH for a medium was determined in medium with maltose in which *C. guilliermondii* cells were incubated (pH 1.92). It is noteworthy that at the same time a further increase in the optical density was observed (Table 2). This proves the ability to develop cells despite such a low pH.

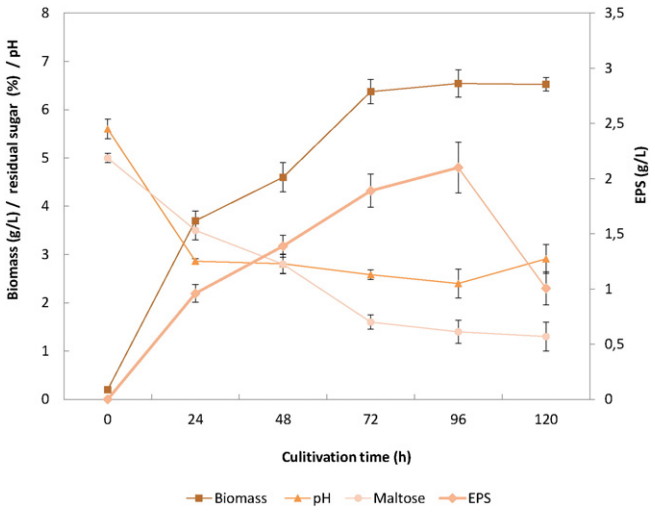

Fig. 1. Time course of EPS synthesis, pH, cell growth and maltose consumption during *Candida famata* cultivation. Values represent the mean ± SD from assays of three samples.

Strong acidification of medium during the synthesis of exopolysaccharides by other yeast genera has been observed in several studies [2,11], wherein the degree of acidification was dependent on the source of nitrogen, and this increased when using inorganic salts. Cho et al. [13] suggested that rapid utilization of ammonium ions, which results in the ejection of protons from yeast cells, may be an explanation for the acidification of the culture environment. It should be emphasized that low pH is a necessary condition for EPS biosynthesis. During the cultivation of *Rh. glutinis* KCTC 7989, continuous neutralization to pH 4.0 was applied. Under such conditions, high biomass yield was achieved (49% higher than in cultivation conditions without any control of pH), while the yield of EPS synthesis was very low [13].

In the presence of sucrose and organic nitrogen sources (peptone yeast extract) in the culture medium, the tested strains of *Candida famata* and *C. guilliermondii* did not reduce the acidity significantly (min. pH 5.4), and simultaneously produced several times less EPS than in the mineral medium [16]. An unfavorable effect of organic nitrogen sources on the synthesis of exopolymers has also been observed during the cultivation of other yeasts, among others, from the *Rhodotorula* genus [13].

3.4. The course of EPS biosynthesis in medium with maltose as the only carbon source

Based on the results obtained from this study, maltose was considered the most preferable carbon source for the production of EPS by *C. guilliermondii* and *C. famata* strains. The characteristics of changes in the amount of EPS depending on the duration of cultivation in media with maltose aimed at optimization of cultivation time. At this stage of the study, the incubation time for both strains was extended to 120 h.

During cultivation of *C. famata* in medium with maltose, the amount of exopolymers increased until the 96 h of incubation (Fig. 1). The highest concentration of extracellular polysaccharides (above 2.10 g/L) was obtained on the fourth d of incubation. In subsequent days, EPS concentration decreased to 1.00 g/L. The biomass yield reached a maximum value in the 96 h of cultivation (6.54 g/L). Subsequently, a slight decrease in the biomass yield was observed. The results of the determination of biomass yield were correlated with the number of cells determined by the plate method and by optical density (data not shown). During cultivation, rapid acidification of the environment was observed during the first 24 h. This was evidenced by the reduction of pH from the initial value of 5.6 to 2.86.

During cultivation, *C. famata* cells did not assimilate total available maltose. After 120 h of cultivation, the final concentration of sugars was still above 1%. Pavlova et al. [2] observed the phenomenon of the lack of use of the overall pool of available sugars by *S. salmonicolor* AL_1 strain during EPS biosynthesis in mineral medium.

The greatest increase in *C. guilliermondii* yeast biomass in medium with maltose was observed during the first 24 h of cultivation (Fig. 2), and this was correlated with OD and the number of cells (cfu/mL) (results not shown). During the next days, biomass yield was increasing and in the 120 h of cultivation reached 7.43 g/L. A decrease in pH was observed on the first d from the initial value of 5.60 to 2.86. The maximum content of EPS (2.98 g/L) was reported in the 72 h of cultivation. Subsequently, the amount of polysaccharides reduced significantly, so that on the fifth day it was estimated at only 1.0 g/L. Rusinova-Videva et al. [15] observed a similar phenomenon of a reduction of EPS amount during cultivation of *C. laurentii*. It is probable that the reduction in the concentration of biosynthesis product may be a result of depolymerization of EPS molecules and inclusion of products into cellular metabolism. Possible confirmation of this hypothesis may be explained by increasing biomass yield after 3 d of cultivation, in which the maximum amount of EPS was found.

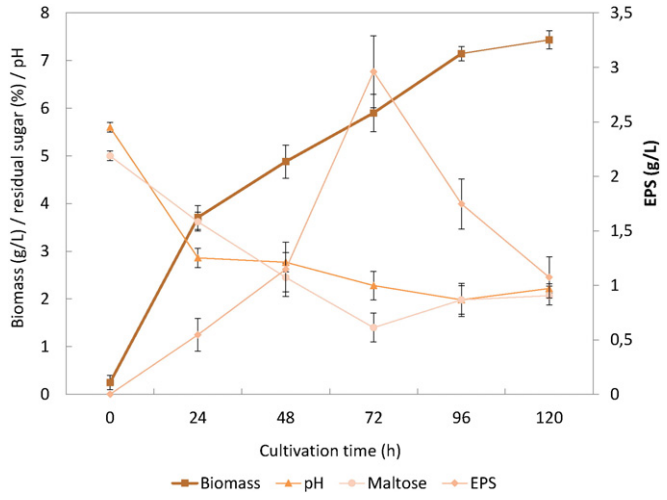

Fig. 2. Time course of EPS synthesis, pH, cell growth and maltose consumption during *Candida guilliermondii* cultivation. Values represent the mean ± SD from assays of three samples.

It was found that the *C. guilliermondii* strain produced more EPS in a shorter time than was the case with the *C. famata* strain. Maximum specific productivity $Y_{p/x}$ of *C. guilliermondii* biomass was 0.505 after 72 h, and it was only 0.321 for *C. famata* (Table 3). Similarly, the highest EPS yield $Y_{p/s}$ from 1 g of maltose was found for the *C. guilliermondii* strain. It should be noted that EPS yield was inversely proportional to the efficiency of biomass yield $Y_{x/s}$. The maximum value of EPS yield ($Y_{p/s}$) was calculated for the same period of cultivation, in which the lowest biomass yield ($Y_{x/s}$) was observed.

It can, therefore, be concluded that, at that particular time, yeast metabolism was clearly directed toward extracellular production of polysaccharides, and not toward the synthesis of intracellular components and biomass production.

The productivity of the *C. guilliermondii* strain was 0.041 g/L/h. This value is comparable to the productivity of the *S. salmonicolor* strain obtained under optimized conditions in medium with sucrose, *i.e.* 0.049 g EPS L/h, estimated from the data published by Pavlova et al. [2].

3.5. Content of carbohydrates in the crude EPS

For the analysis of total sugars in EPS, those which were precipitated after the cultivation of both strains in medium with maltose and sucrose were selected. Crude exopolysaccharides are white water-soluble powders. Exopolysaccharides of both strains obtained in medium with sucrose were characterized by a similar content of carbohydrates: 49.7 ± 5.9 g in 100 g of EPS from *C. famata* and 49.3 ± 2.2 g in 100 g of EPS from *C. guilliermondii*. A beneficial effect of maltose was observed on the sugar content in the EPS. EPS produced by *C. famata* contained 61.5 ± 4.3% carbohydrates. Most sugars, above 65.4 ± 4.3%, were found in EPS produced by *C. guilliermondii*. It had previously been shown [2,15] that proteins and minerals constitute the remaining components of preparations. Over 12% of proteins and about 4% of mineral salts are contained in EPS produced by *C. laurentii* AL_{62}, and the preparation obtained after *S. salmonicolor* Al_1 cultivation contains 5.3% proteins and 4.54% mineral salts [2].

The carbon source significantly affected the carbohydrate content in extracellular polysaccharides derived from the tested strains. However, the content of sugars was lower than their cellular content in the extracellular polymers obtained after cultivation of other strains. Over 83% and 85% of sugars were contained in EPS derived from *Cr. laurentii* AL_1 [15] and *Rhodotorula acheniorum* cultivation [14], respectively. The highest content of saccharides, exceeding 90%, was determined in crude EPS preparation produced by *S. salmonicolor* AL_1 (above 90%) [2].

Table 3
The effect of time of cultivation on EPS specific production, EPS yield and biomass yield by *Candida* strains in mineral medium containing maltose.

Strain/time of culturing		Specific production ($Y_{p/x}$)	Consumed maltose g/L	EPS yield ($Y_{p/s}$)	Efficiency of biomass yield ($Y_{x/s}$)
C. famata	24 h	0.259	15.00	0.064	0.246
	48 h	0.301	22.10	0.063	0.208
	72 h	0.296	34.00	0.056	0.187
	96 h	0.321	36.10	0.058	0.181
	120 h	0.154	37.00	0.027	0.176
C. guilliermondii	24 h	0.148	13.80	0.040	0.269
	48 h	0.235	25.50	0.045	0.191
	72 h	0.505	36.00	0.083	0.164
	96 h	0.243	30.20	0.058	0.237
	120 h	0.145	29.30	0.037	0.253

3.6. Crude EPS as a carbon source for producer strain

During the last hours of incubation a reduction of crude EPS amounts was observed for both strains. At the same time, reducing sugars content was increased, which is an indirect proof of the occurrence of the depolymerization of EPS. This may be due to the activity of enzymes released into the culture medium. During stationary phase, the cells undergo lyse and leakage of the cytoplasm occurs. It can include EPS cutting enzymes. However, autolysis of cells may not explain the phenomenon of a decrease in the contents of EPS, since the following days of incubation showed an increase in biomass. However, the assimilation of exopolymers as a carbon source by the yeast must be preceded by the activity of enzymes that degrade crude EPS to sugars. This should constitute indirect evidence of the presence of corresponding enzyme activity. Therefore, an attempt was made to check the possibility of the use of crude exopolysaccharides as carbon sources by *C. famata* and *C. guilliermondii* cells. The conditions of this experiment are presented in the Materials and methods section.

During Bioscreen C and flask cultures, an increase in Candida strain cells was observed. The cultures were distinguished by the long duration of the lag-phase. On average, the beginning of the log-phase was observed in 42 h of incubation for the *C. famata* strain and four hours later for the *C. guilliermondii* strain (Table 4). The specific growth rate (μ) of *C. famata* in medium containing crude EPS as a sole carbon source was 0.0068 h^{-1}. For the latter strain, the value of this parameter averaged 0.0138 h^{-1}.

The number of viable *C. guilliermondii* cells (determined by plate methods) during 96 h of incubation increased from an initial value of 1.7×10^3 cfu/mL to 3.85×10^7 cfu/mL. The increase in the number of *C. famata* cells during the period of cultivation was also significant. *C. famata* cells can also utilize self-produced EPS.

Table 4
The impact of EPS as a carbon source in mineral medium for the course of cultivation *C. famata* and *C. guilliermondii* strains.

Type of culture	Growth parameters	*C. guilliermondii*	*C. famata*
Bioscreen C culture	Length of lag phase h	46 ± 1	42 ± 0.5
	Length of log phase h	24 ± 1	28.5 ± 1
	OD_i in log phase	0.810 ± 0.056	0.918 ± 0.044
	OD_f in log phase	1.129 ± 0.048	1.117 ± 0.038
Flask culture	$\mu(t)$ h^{-1}	0.0138 ± 0.0007	0.0068 ± 0.0005
	log CFU_i/mL	3.23 ± 0.11	3.10 ± 0.09
	log CFU_f/mL	7.58 ± 0.09	7.59 ± 0.10
	Δ_{f-i} log CFU/mL	4.35 ± 0.10	4.49 ± 0.09

The experiment showed that crude EPS (derived from mineral medium with maltose) can be a source of carbon for the strains that produce it. It can be concluded that *C. famata* and *C. guilliermondii* strains can synthetize appropriate enzymes that degrade EPS into assimilated monosaccharides. The examined crude exopolysaccharides are a food reservoir for the yeast cells.

From the literature data there is a known glucoamylase activity for *C. famata* [20]. Glucoamylase (EC 3.2.1.3) consecutively hydrolyzes α-1,4 glycosidic bonds from the non-reducing ends of starch, resulting in the production of glucose. This may suggest the presence of α-1,4 glycosidic bonds in EPS produced by *C. famata*. Future studies should involve the analysis of the composition or structure of exopolysaccharides.

4. Conclusion

C. famata and *C. guilliermondii* strains isolated from kefirs exhibited the ability to biosynthesize extracellular polymers.

The amount of crude exopolysaccharides varies significantly depending on the source of carbon. Maltose was the preferred carbon source for the synthesis of EPS by both Candida strains, in contrast to sucrose which was considered by other authors to be the most suitable carbon source for the production of EPS by Rhodotorula and Cryptococcus strains. In mineral medium containing various carbohydrates, but the same carbon to nitrogen proportions, biosynthesis of polymers varied; therefore, C:N did not have a direct effect on EPS biosynthesis. The highest EPS yield, $Y_{p/s}$ (0.083), from 1 g of maltose was found for the *C. guilliermondii* strain. Sorbitol and glycerol were considered unfavorable carbon sources in the synthesis of EPS.

The crude exopolysaccharides of both strains obtained in medium with sucrose were characterized by a similar content (above 49%) of carbohydrates. The contribution of carbohydrate in the precipitated preparation of EPS was higher during supplementation of maltose as compared to sucrose. The sugar content in EPS from *C. famata* increased by 24% and from the *C. guilliermondii* strain by 33%.

The phenomenon of the strong acidification of mineral medium during the synthesis of EPS was confirmed for both strains. In each post-culture medium, a significant increase in the active acidity of the culture environment was reported and the lowest pH of medium (pH 1.92) was determined in *C. guilliermondii* medium with maltose. A further increase in the optical density was observed, which proves the ability of the cells developed despite such a low pH.

The maximum contents of EPS were reported on the third day of *C. guilliermondii* cultivation and on the fourth day of *C. famata* cultivation in mineral medium with maltose. Subsequently, the amount of polysaccharides reduced significantly. At the same time, reducing sugars content was increased which is an indirect proof for the occurrence of the depolymerization of EPS. Therefore, an attempt was made to check the possibility of the use of crude exopolysaccharides as carbon sources by *C. famata* and *C. guilliermondii* cells. In the separate experiment we showed that crude EPS can be only one source of carbon for the strains that produce it. The examined crude exopolysaccharides are a food reservoir for the yeast cells. The duration of cultivating in mineral medium are a significant factor in the biosynthesis of exopolysaccharides by *C. guilliermondii* and *C. famata* strains isolated from kefirs.

Conflict of interest

No conflicts of interest between the researchers in this topic of research.

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