EFFECT OF DIFFERENT CARBON SOURCES ON BIOSYNTHESIS OF EXOPOLYSACCHARIDE FROM ANTARCTIC STRAIN CRYPTOCoccus LAURENTII AL_{62}

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ABSTRACT
Isolate AL_{62} isolated from Antarctic soil was identified as Cryptococcus laurentii AL_{62} based on its morphological, cultural and physiological properties and was selected as an active producer of exopolysaccharide. Different carbon sources (pentoses, hexoses and oligosaccharides) were investigated for biosynthesis of biopolymer and sucrose was chosen as the most suitable and available carbon source. The time course of exopolysaccharide synthesis, biomass and pH on different sucrose concentration (30, 40, 50 g/L) were studied. The heteropolysaccharide was composed of the following monosaccharides: xylose, 45.2 %; mannose, 33.6 %; glucose, 18.4 %. It was characterized by polydispersity of the polymer molecule, 60 % of it having molecular mass of 8000 Da.


Keywords: Antarctic yeast strain Cryptococcus laurentii, biosynthesis, exopolysaccharide

Introduction
The Antarctic microorganisms have recently attracted considerable attention for their metabolite adaptation at extreme Antarctic conditions and for practical reasons with the possibility for study of their biological potential for obtaining biologically active substances with useful properties (3, 6, 11, 15, 18, 19, 21, 31).

The genus Cryptococcus is the most predominant yeast group in the Antarctic. In this genus Cr. laurentii and Cr. albidus are the most popular species. Several new species of Vishniac have been isolated from various habitats and sites of collection in the Antarctic (29). A large number of scientists have extended their culture collections from yeasts of the Cryptococcus genus by isolation from Antarctic soil samples the strains Cryptococcus vishniacii (25, 26, 30), Cryptococcus friedmannii (29), Cryptococcus antarcticus and Cryptococcus albidosimilis (27, 28), Cryptococcus socialis and Cryptococcus consortions (24), Cryptococcus victoriae (9), Cryptococcus adeliensis, Cryptococcus albidus, Cryptococcus laurentii (12, 21). The moss, lichens, bird samples and moulting feathers of penguins from Antarctic are sources for isolation of strains from this genus: Cryptococcus nyarrowii (22), Cryptococcus starzelliae and Cryptococcus victoriae (23), Cryptococcus laurentii, Cryptococcus flavus, Cryptococcus albidus (14).

From Antarctic yeasts from the Cryptococcus genus were selected producers for synthesis of enzymes, polysaccharides, carotenoids, lipids, bioremediation agents (7, 10, 13, 15, 16, 17, 29, 31).

The aim of this paper was to study the biosynthesis of the Antarctic Isolate AL_{62} exopolysaccharide in different carbon sources and to study the time course of the process with sucrose.

Materials and Methods

Microorganism
Cryptococcus laurentii AL_{62} was selected as a suitable producer of exopolysaccharide (20). Samples (soil) were taken from different sites of Livingston Island by the Bulgarian Antarctic Expedition in the summer of 2006-2007. The samples were suspended in sterile water and after suitable dilution they were plated on malt agar. The cultivation was carried out at 4 °C for 3 to 14 days. The isolated colonies were reinoculated several times for purity, maintained on malt slant agar and stored at 4 °C. The yeasts studied belonged to the psychrophilic yeast collection isolated from Antarctic samples.

Media and Growth Conditions
The fermentation medium contained (g/l): sucrose, 40; (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, 2.5; KH\textsubscript{2}PO\textsubscript{4}, 1.0; MgSO\textsubscript{4}.7H\textsubscript{2}O, 0.5; NaCl, 0.1; CaCl\textsubscript{2}.2H\textsubscript{2}O, 0.1; yeast extract, 1.0. The pentoses (xylose, ribose, arabinose), hexoses (galactose, glucose, fructose, mannose and rhamnose) and oligosaccharides (raffinose, trehalose and sucrose) were added in a 10 g/L concentration. Sucrose was chosen as a suitable carbon source and studied in different concentrations (g/L): 30, 40, and 50. The initial pH was adjusted to pH 5.3. The inoculum was obtained on a rotary shaker (220 rpm) in 500 ml Erlemeyer flasks containing 50 mL medium of Sabouraud medium (Merck, Germany), on a shaker at 22 °C for 48 h. The fermentation medium was inoculated with 10.0% w/v inoculum. The cultivation was carried out in 500 mL Erlemeyer flasks containing 50 mL of the medium.
Fig. 1. Quantities of EPS and biomass synthesized by *Cr. laurentii* AL-62 on different carbon sources (at 10 g/L concentration): pentoses (A), hexoses (B) and oligosaccharides (C).

Fig. 2. Time course of polysaccharide synthesis (dashed line), biomass (dotted line) and pH (solid line): culture medium with 30 g/L (A), 40 g/L (B) and 50 g/L (C) sucrose, at 22 °C.
on a rotary shaker (220 rpm) at 22 °C for 120 h. The cells were collected by centrifugation. The exopolysaccharides in the supernatant were precipitated with two volumes of cold absolute ethanol, held at 4 °C overnight and then centrifuged at 6000 g for 30 min, washed with ethanol and dried.

**Analytical methods**

The yield of exopolysaccharides and the dry biomass were determined by the weight method after drying to constant mass at 105 °C.

The total carbohydrate amount in the crude EPS was determined using the phenol-sulphuric acid method (4). The protein amount in the solution of non-hydrolysed polysaccharide was determined according to the Lowry method. The ash content was estimated after calcination for 2 h and glowing the polymer at 550 °C for 3 h.

Neutral sugars were measured as alditol acetates after hydrolysis of samples. The polysaccharides (20 mg) were pretreated with 2M trifluoroacetic acid (TFA) for 3 h at 120 °C before conversion to alditol acetates according to the method developed by Blakeney et al. (2). The monosaccharide composition of the polysaccharides was assessed on a gas-mass chromatograph system – 6890 GC System Plus/ 5793 MassSelective Detector (Hewlett Packard) with an SP-2380 capillary column (0.2 μm film, 0.25 mm i.d.×30 m, Supelco). Using the temperature program: column temperature for 3 min, then 5 °C/min to 250 °C; detector temperature 280 °C; helium as carrier gas at 1ml/min. The column was calibrated using the p-82 Shodex 500 column (7.8 × 300 mm; Waters). The study of the hexose effect on the epS synthesis by the producer on 1Α glucose or produce acids from glucose; it did not form starch-like compounds. It grew on a vitamin-free medium containing 50% glucose was positive and 10% NaCl and 5% glucose was negative.

**Results and Discussion**

In a previous study on the metabolic potential of Antarctic yeast for exopolysaccharide synthesis, Cryptococcus sp. Al62 showed initial EPS quantity of over 4.5 g/l and was selected as an active biopolymer producer (20).

The isolate Al62 was related to Cryptococcus laurentii Al62.

The results of the study of the Cr. laurentii Al62 strain’s physiological properties for exopolysaccharide and biomass biosynthesis through utilization of different carbon sources – pentoses, hexoses and oligosaccharides in 1.0% concentration, are shown in Fig. 1. The EPS biosynthesis by the producer on a xylose, ribose and arabinose containing medium was within the 1.5 – 1.7 g/L range, and the amount of accumulated biomass was 3.0 g/L. Xylose was a good carbon source transformed by the culture into 1.7 g/L biopolymer and amount of biomass (3.0 g/L) compared to the biomass obtained on a arabinose (3.11 g/L) and ribose containing medium – 2.87 g/L, (Fig. 1A). The study of the hexose effect on the EPS synthesis by the strain showed that galactose, glucose, fructose, mannose and rhamnose were transformed into a polysaccharide, and as a result 3.1 g/L of exopolysaccharide were obtained with mannose. The oligosaccharides raffinose, fructose and rhamnose were transformed by Cr. laurentii Al62 into a polymer, the quantity of which exceeded 2.4 g/L, the biomass of the trehalose and sucrose containing media was over 3.00 g/L, and when raffinose was used, it was 2.5 g/L (Fig. 1C). The isolate’s preferences for a carbon source for biopolymer and biomass synthesis were related to its physiological characteristics and taxonomic affiliation.

**TABLE 1**

<table>
<thead>
<tr>
<th>Carbohydrate, %</th>
<th>Protein, %</th>
<th>Ash, %</th>
<th>Monosaccharide, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Xylose</td>
</tr>
<tr>
<td>83.7</td>
<td>12.6</td>
<td>3.7</td>
<td>45.2</td>
</tr>
</tbody>
</table>

BIOTECHNOL. & BIOTECHNOL. EQ. 25/2011/4, SUPPL.
Sucrose was used for study of the time course of the biosynthetic process by *Cr. laurentii* AL\(_{62}\) at 22 °C. The EPS synthesis, the accumulation of biomass and the variation of pH during the process, were assessed at concentrations of 30, 40, 50 g/L (Fig. 2).

The amount of produced exopolysaccharide during cultivation in medium containing 30 g/L sucrose reached a maximum (3.97 g/L) 48 h from the beginning of the fermentation process. After that the target product decreased probably as a result of depolymerization of the EPS molecule and inclusion of the products in the metabolism of the producer because of finishing of the main carbon source. In this connection a slight increase of biomass near 5.55 g/L at 72 h was observed. Similar results were also obtained for the exopolysaccharide yield at cultivation medium with 40 g/L sucrose, EPS – 4.6 g/L at 48 h and biomass 6.36 g/L at 96 h. There was a proportional dependence between the amount of synthesized EPS and the obtained biomass at 30 g/L and 40 g/L sucrose.

The maximum EPS synthesis of 4.73 g/L was observed on a culture medium with 50 g/L sucrose at 72 h when the biomass was 6.6 g/L. A typical feature of the production of EPS from yeasts is the significant pH change, which proves to be a regulating factor in the EPS biosynthesis (1, 5). In the course of its metabolism, *Cr. laurentii* AL\(_{62}\) strain changed the culture medium pH from the initial pH 5.3 to pH 2.2 after 24 h and these pH values were preserved until fermentation was complete. The strain *Cr. laurentii* AL\(_{62}\) had good biosynthetic capacity for producing EPS in the medium with 40 g/L and 50 g/L sucrose, and it was observed that the highest quantity of biopolymer was obtained at 48 h and at 72 h respectively.

The chemical and monosaccharide composition of the crude EPS are shown in Table 1. It contains 83.7% carbohydrate, 12.6% protein, and 3.7% ash. The newly synthesized microbial carbohydrate is a heteropolysaccharide of the following monosaccharide composition: xylose – 45.2%, manose – 33.6%, glucose – 18.4%, and other carbohydrates – 2.7%. HPSEC was used to establish the heterogeneity of the EPS which contained several fractions with molecular masses ranging from 1.24e+06 Da to 210 Da, 60% of the polysaccharide having molecular mass of 8 000 Da (Fig. 3).

Conclusions
The strain *Cryptococcus laurentii* AL\(_{62}\) was selected from Antarctic samples for biosynthesis of polysaccharide. The data from its biosynthesis showed the strain’s preferences for a carbon source and the potential for synthesis of biopolymer on medium with sucrose at a concentration of 40 g/L.

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REFERENCES