# STUDIES OF ANTARCTIC YEAST ISOLATES FOR EXOPOLYSACCHARIDE SYNTHESIS

S. Rusinova-Videva[1], K. Pavlova[1], R. Metcheva[2]
[1]Department of Microbial Biosynthesis and Biotechnology, Institute of Microbiology, Bulgarian Academy of Sciences, Plovdiv, Bulgaria
[2]Institute of Zoology, Bulgarian Academy of Sciences, Sofia, Bulgaria
Correspondence to: Snezhana Rusinova-Videva
E-mail: jrusinova@abv.bg

## ABSTRACT

*From Antarctic samples were obtained yeast isolates that were investigated for exopolysaccharide synthesis. The screening process revelated that significant number of psychrophilic yeasts had the ability to produce biopolymers.Perspective strains were selected. A physico-chemical analysis of Antarctic samples was made.*

**Keywords:** Antarctica, polysaccharides, psychrophylic yeast

## Introduction

The Antarctic continent is characterized with unique combination of extreme environment conditions – low temperature and humidity, high winds, extended light and dark periods, nutrient and water deficiency (11). The land areas of the Antarctic are or constantly frozen or they thaw only for several weeks during the summer (2,6). These natural habitats include cold soils, plants (mosses, lichens, grasses), lakes, sediments, cold-blooded animals (fishes). A great number of microorganisms (fungi, bacteria, yeasts, algae) are capable of growing in extreme environments (4). Commonly, the eukaryotes are psychrophiles, acidophiles, alkaliphiles, piezophiles, xerophiles, and halophiles, which respectively thrive at low temperatures, low pH, high pH, under extreme pressure, desiccation, and salinity (12). Extremophilic microorganisms have recently attracted considerable attention not only for their metabolite adaptation at extreme conditions, but also for their biotechnological potential (1,5). A progress in the research of Antarctic microorganisms is made by the creation of culture collections and screenings for perspective strains, producing biologically active compounds – enzymes (7,8,14) polysaccharides (9,10), lipids (3,16), etc. The interest for biotechnological production of polysaccharides is determined by the possibility of different microorganisms to synthesize exopolysaccharides, applied in the food, pharmaceutical, cosmetic, and other industries as emulsifiers, stabilizers, binding and gelling agents, coagulants, lubricants, film forms, thickening and suspending agents. The Antarctic yeasts are insufficiently studied as producers of exopolisaccharides with new functional properties and applications.

In this paper we present the results from physical and chemical characterization of Antarctic soil and moss samples, from which yeast isolates were obtained and perspective strains, producing new exopolisaccharides were selected.

## Materials and methods

*Antarctic samples from Livingston, Antarctica and microorganisms.* Livingston Island is the second island among the South Shetlands located between 62° 27′ - 62° 48′ S and 59° 45′ - 61°15′ W of the northern sea coast of the Antarctic Peninsula. Samples (soil and moss) were taken from different sites of Livingston Island by the Bulgarian Antarctic Expedition in the summer of 2006-2007 and 2007-2008. 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.

*Exopolysaccharide (EPS) production.* The fermentation medium contained (g $l^{-1}$): sucrose, 40; $(NH_4)_2SO_4$, 2.5; $KH_2PO_4$, 1.0; $MgSO_4.7H_2O$, 0.5; NaCl, 0.1; $CaCl_2.2H_2O$, 0.1; yeast extract, 1.0. The initial pH was adjusted to pH 5.3. The

inoculum from psychrophilic isolates were obtained in 100 ml Erlenmeyer flasks containing 50 ml of Sabouraud medium (Merck, Germany), on a rotary shaker at 220 rpm at 22°C for 48 h.The fermentation medium was inoculated with 10.0% w/v inoculum. The cultivation was performed at 22°C for 120 h with shaking at 220 rpm. 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.

*Analyses.* The yield of exopolysaccharides and the dry biomass were determined by the weight method after drying to constant mass at 65°C and 105°C. The viscosity was determined by the method of Stoks, 20°C (13).

The IR spectra of exopolysaccharide were implemented using KBr tablets on a spectrometer Nicolet Avatar 330 FT-IR, Thermor Electron Corporation, Madison, USA.

## Results and Discussion

The Bulgarian scientific research expeditions on Livingston Island provided us with Antarctic soil and moss samples. The structural and functional characteristics of the samples were determined, since these features were preconditions for the existence of microorganisms and uncovered the potentiality for their isolation. The results from the physical and chemical analysis of the Antarctic soil and moss samples are shown in **Table 1**.

The structure of soil sample 1 was coarse-grained and the sample contained plant tissues such as small roots and leaves. Sample 2 was sandy structured with the presence of small roots. Sample 3 was definitely fine-grained and sandy. The soil samples were characterized with neutral pH values, at which microorganisms display the highest life activity. The humidity of the samples was determined to be low, which limited the presence and growth of microorganisms. The number of the isolates selected from the three samples was as follows: eight isolates from sample 1, five isolates from sample 2, and three isolates from sample 3. The chemical analysis of the soil samples, regarding carbon and nitrogen content, showed that these basic nutrient sources are available in small quantities. This fact is explained by the extreme environment conditions on the continent, which limit flora and fauna diversity. The ratios of carbon and nitrogen source in the soil samples had close values: 10:1, 12:1, and 11.9:1 respectively. The high phosphorus content of sample 3 is probably the reason for the small number of isolates obtained.

Sixteen isolates were selected from the Antarctic moss samples. The moss samples were characterized with up to ten folds higher carbon content and two folds higher nitrogen content in comparison to the soil samples – the ratio C:N is 61:1 in sample 4, and 41:1 in sample 5. The higher concentration of carbon and nitrogen and the higher humidity of the plant samples explain the higher percentage of yeasts isolated. Additionally, nine isolates were selected from Antarctic samples (soil and penguin feather) taken from the Polish base, situated on King Georgia Island. Totally, 41 psycrophilic yeast isolates were collected.

The metabolite potential of the isolated yeasts for biosynthesis of exopolysaccharides was studied. Eleven strains, producing exopolysaccharides were selected by submerged cultivation on a synthetic nutrient medium, containing 4 % sucrose. The potential for exopolysaccharides biosynthesis was evaluated by the yield of polysaccharides, by the measured viscosity, and by the quantity of accumulated biomass (**Fig. 1**).

The concentration of produced biopolymers varied in the range of 4.00 $gL^{-1}$ to 5.15 $gL^{-1}$. The content of biomass was in the range of 2.40 % to 4.22 %, and the viscosity was from 1.97 Pa.s to 2.77 Pa.s. According to the results, the strains, producing over 4.70 $gL^{-1}$ exopolysaccharides were considered to be of interest. The first six strains synthesized an almost equal quantity of biopolymers. For this reason they will be used for further selection of a perspective strain after determination of some physical and chemical parameters. The biomass concentrations were significantly lower than exopolysaccharides concentrations. Probably, this was due to biotransformation of carbon source to biopolymers in a higher degree in comparison to its utilization as energy supplement for cell growth. The measurement of the supernatant viscosity by the method of Stocks provided us with preliminary information for the reologic properties of the exopolysaccharides, produced by the tested isolates. The results showed no correlation between the three parameters determined, probably the isolates taxonomically belong to different genera and species. During the cultivation of the psychrophilic yeast isolates on a nutrient medium with an initial pH value of 5.3, a decrease of pH was detected at hour 24 of the process – pH values varied in the range of 1.92 to 2.60. The process was under its own control, which ensured sterility of the fermentations.

**TABLE 1**

Characterization of Antarctic siol and moss by Livingston Island

| № | Livingston Island | pH | Humidity, % | Carbon, g $kg^{-1}$ | Nitrogen, g $kg^{-1}$ | Phosphorus, g $kg^{-1}$ |
|---|---|---|---|---|---|---|
| 1. | 62°38′14.6″ S 17м. н. в. 60°21′36.0″ W | 7.36 | 0.6 | 15.1 ± 1.0 | 1.45 ± 0.13 | 1093 ± 170 |
| 2. | 62°38′49.1″ S 36м. н. в. 60°22′18.2″ W | 7,06 | 1,3 | 43.0 ± 3,2 | 3,52 ± 0.31 | 1576 ± 244 |
| 3. | 62°38′35,8″ S 43м. н. в. 60°22′14.4″ W | 6.12 | 0.8 | 43.7 ± 3.2 | 3.67 ± 0.33 | 2272 ± 352 |
| 4. | 62°40′09″ S . 60°24′08″ W | 7.15 | 2.1 | 472 ± 34 | 7.6 ± 0.7 | 1232 ± 191 |
| 5. | 62°40′09,8″ S 60°24′08,1″ W | 7.21 | 3.2 | 259 ± 19 | 6.3 ± 0.6 | 2436 ± 223 |

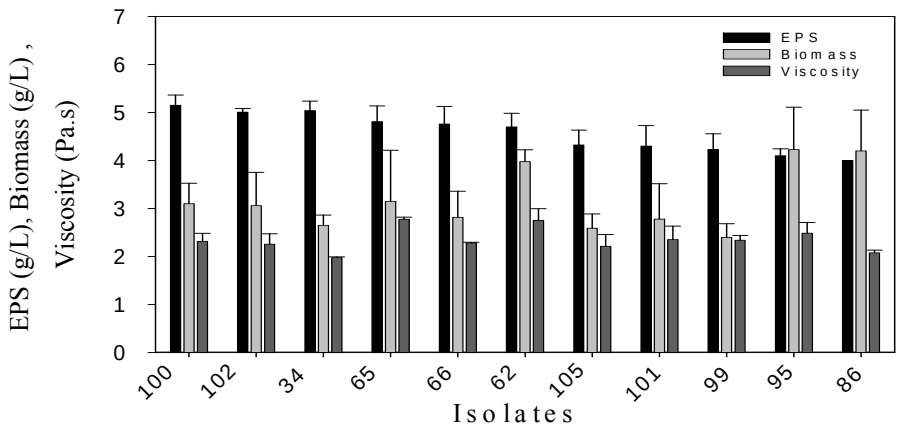


**Fig. 1**. Biosyntesis of exopolysaccharide, biomass and Viscosity from different psychrophilic yeast Isolates.

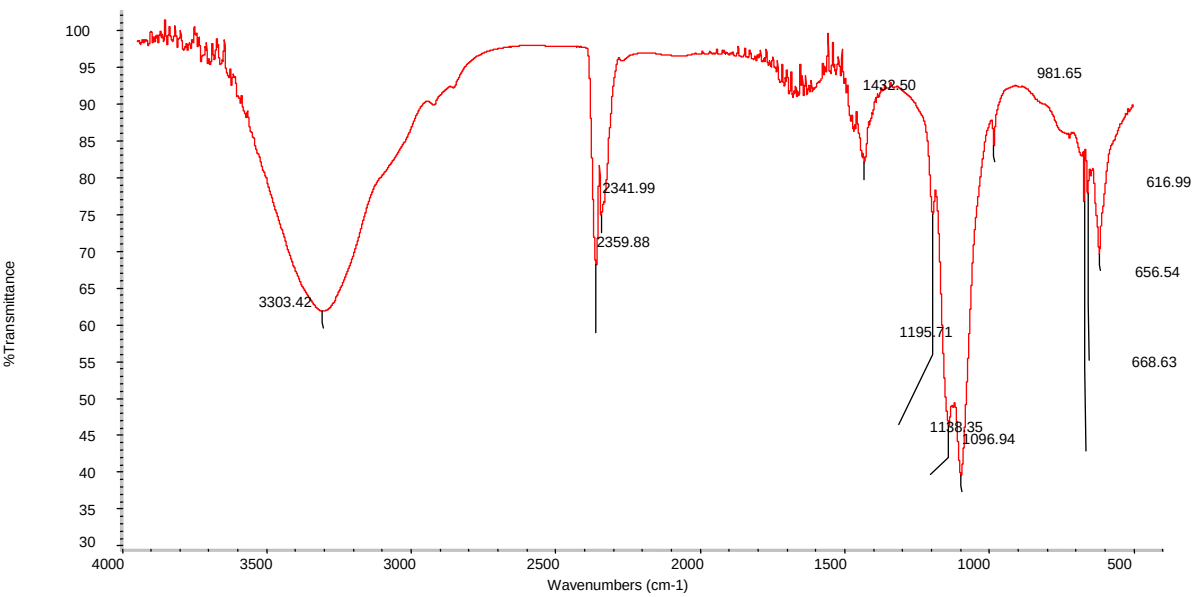


**Fig. 2**. IR spectra of polysaccharide from psyhrophilic yeast Isolate 100

We observed in the IR spectra of exopolysaccharide of yeast isolate$_{100}$ characteristic absorption bands typical of natural polysaccharides. The band at 2900-3300 $cm^{-1}$ was distinctive of the -$CH_2$ group, and at 3300-3600 $cm^{-1}$ of the presence of –OH groups, involved in the formation of inter-molecular hydrogen bonds. The band we observed at 1648-1654 $cm^{-1}$ according Zhang et al., 2001 (15) is due to the in plane deformation of the water molecule. This water is the strongly bound water of crystallization.

In the neutral Antarctic samples from soil and moss with low humidity and different quantities of the basic carbonic and nitric nutrient sources, there exist conditions for growth of microbic populations from which we have isolated and selected psychrophilic yeasts with high biological potential for synthesis of exopolysaccharides.

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