THE STEPHAN ANGELOFF INSTITUTE OF MICROBIOLOGY

BULGARIAN ACADEMY OF SCIENCES

MEDICAL UNIVERSITY OF SOFIA

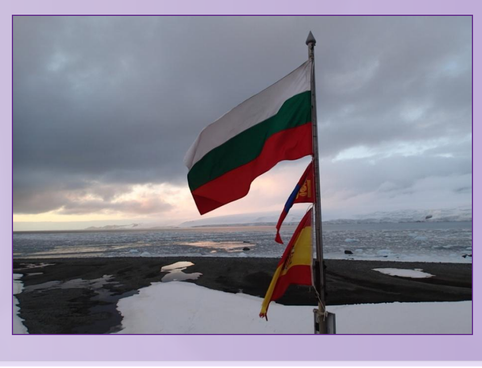
UNIVERSITY OF PLOVDIV "PAISII HILENDARSKI"

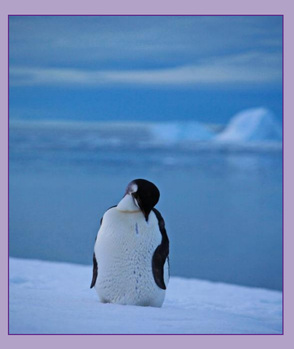

# Evaluation of the antineoplastic activity of Antarctic yeast *Sporobolomyces salmonicolor* grown at different culture conditions


**Rusinova-Videva S.[1], Hristova D.[2], Zaharieva M. M.[3], Nachkova S.[4], Kambourova M.[5], Najdenski H.[3], Konstantinov S.[2]**

*[1]The Stephan Angeloff Institute of Microbiology - Bulgarian Academy of Sciences, Department Biotechnology, Plovdiv 4000, Bulgaria*
*[2]Medical University of Sofia, Faculty of Pharmacy, Department of Pharmacology, Pharmacotherapy and Toxicology, Sofia 1000, Bulgaria*
*[3]The Stephan Angeloff Institute of Microbiology - Bulgarian Academy of Sciences, Department of Infectious Microbiology, Sofia 1113, Bulgaria*
*[4]University of Plovdiv "Paisii Hilendarski", Department of Analytical Chemistry and Computer Chemistry*
*[5]The Stephan Angeloff Institute of Microbiology - Bulgarian Academy of Sciences, Department of General Microbiology, Sofia 1113, Bulgaria*





Cancer is in the top three causes of morbidity and mortality worldwide especially in the developed countries. Only in Europe cancer represents the second most important cause of death and morbidity with more than 3.7 million new cases and 1.9 million deaths registered every year which represents about 25% of the global cancer burden. The most common neoplasms locations were breast (12.4 %), colorectal (11.8 %), lung (11.1 %), prostate (10.6 %) and bladder cancer (4.7 %) (WHO, 2020).

Due to the heterogeneous character of the malignant diseases and the constantly arising problems with systemic toxicity and resistance towards numerous clinically applied chemotherapeutics, there is an urgent need for continuous search for new antineoplastic agents. In the last decade the inhibition of cancer cells by natural compounds and extracts attracted much attention. Microbial metabolites as antineoplastic agents and tools for modulation of apoptosis signal transduction represents an attractive therapeutic concept due to the specific targeting of cancer cells and unlike chemical inhibitors do not lead to significant toxic side effects.

During the last several years intensive investigations on Antarctic microflora revealed an ability of psychrophilic yeasts to synthesize new substances with intriguing functional properties. However, the scarcity of information about the synthesis of biologically active substances with antineoplastic effect from Antarctic yeasts and social significance of malignant tumor formations affecting millions of people worldwide determined our interest in evaluation of the antiproliferative and pro-apoptotic potential of several extracts obtained from the psychrophilic strain *Sporobolomyces salmonicolor* $AL_{36}$ cultivated under different conditions.

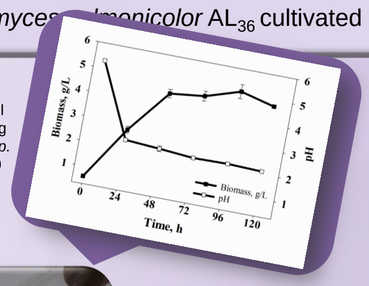



**Fig.1**. Time course of cell growth and pH monitoring during of cultivation by *Sp. salmonicolor* $AL_{36}$ at 220 rpm, 22°C, 120h.

During the stationary phase biomass was almost unchanged and 5.0 g/L were registered on 96th h. The determined productivity was 0.05 g/L/h (**Fig.1**).

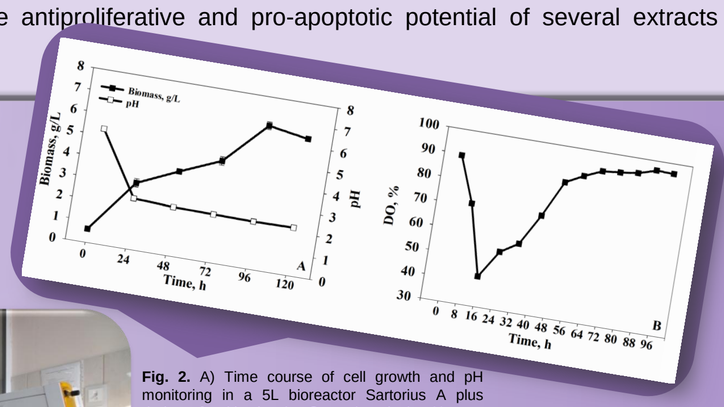



**Fig. 2.** A) Time course of cell growth and pH monitoring in a 5L bioreactor Sartorius A plus during of cultivation by *Sp. salmonicolor* $AL_{36}$, at 400 rpm,22°C, 1L/L/min, 120h; B) Time course of dissolved oxygen in the culture medium in the cultivation by *Sp. salmonicolor* $AL_{36}$ in a bioreactor

Cultivation in the bioreactor was characterized by a longer exponential phase (96 h) compared to flask cultures (**Fig. 2A**). At the end of the exponential phase, an optimal amount of accumulated biomass was observed - above 6.0 g/L (≈6,4 g/L). Performance of the fermentation process in a bioreactor showed that maximum biomass was accumulated after 96 h cultivation. Then the amount did not change significantly. Based on the results obtained, the productivity of the strain was determined as 0.066 g/L/h.

Submerged cultivation by *Sporobolomyces salmonicolor* $AL_{36}$

Bioreactor cultivation by *Sporobomyces salmonicolor* $AL_{36}$

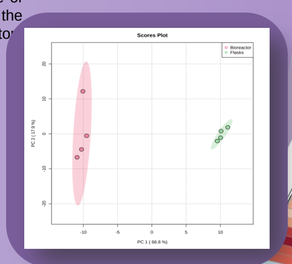



**Fig. 3**. PCA scores plot between the first and the second principal components with the explained variances in brackets (Bioreactor culture-red; flask culture-green).

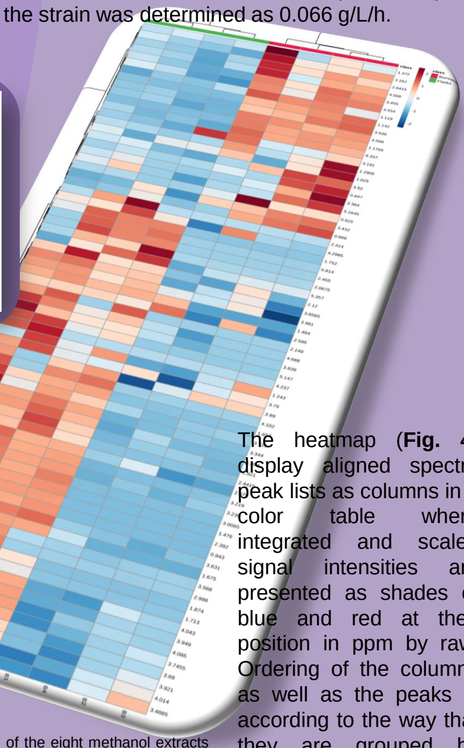


**Fig.4**. Heatmap of the eight methanol extracts spectra with autoscaled features (bioreactor culture-red; flask culture-green).

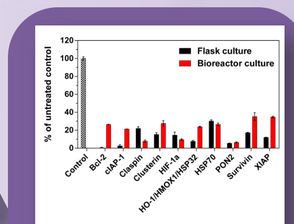
The heatmap (**Fig. 4**) display aligned spectra peak lists as columns in a color table where integrated and scaled signal intensities are presented as shades of blue and red at their position in ppm by raw. Ordering of the columns as well as the peaks is according to the way that they are grouped by similarity shown as hierarchical tree sideward.

The detail view reveals particular variances in the two groups of NMR spectra – some of the signals were more intense in the spectra of extracts of flask culture while others in the spectra obtained from bioreactor culture. Spectral peaks of bioreactor cultivated biomass dominated the interval below 2 ppm, attributable to amino acids and organic acids and they were less prominent in the region of the sugars than in the spectra of flask cultivation. Overall differences were visualized by the applied multivariate statistical methods (**Fig.3 and 4**). PCA score plot represent similarity/dissimilarity between spectra by the closeness of points in two-dimensional space, but do not provide information for discriminative features (**Fig. 3**).

The antineoplastic effect of the accumulated biomass was evaluated on a panel of malignant cell lines originating from different neoplastic diseases, including tumour and haematological malignancies and one non-malignant cell line derived from normal mouse fibroblasts (SKW-3,HuT-78,MJ,RPMI-8226 and CCL-1). The $IC_{50}$ values calculated from the MTT assay varied depending on the cell line.

A comparative examination of the metabolic profiles of the two extracts tested revealed differences in the synthesized molecules corresponding to different cytotoxicity *in vitro* on malignant cell lines. The median inhibitory concentration of each extract determined by the MTT test was used as a parameter for evaluating the antiproliferative effects. Most sensitive to the *in vitro* effect of the extract of *Sp. salmonicolor* $AL_{36}$ cultivated in flasks was the cell line SKW-3 (T-cell leukemia, derivative of KE-37)- $IC_{50}$ = 35.3μg/ml, while the bioreactor biomass extract was most cytotoxic for RPMI-8226 (multiple myeloma) cells - $IC_{50}$ = 28.27μg/ml.

The data from the proteome analysis showed that both yeast extracts posses a strong potential to inhibit important anti-apoptotic proteins which reveals a mode of action related to induction of apoptosis and inhibition of cellular proliferation and migration.




| Proteins | Flask culture | Bioreactor culture |
|---|---|---|
| Control | 100.00 ±1.95 | |
| Bcl-2 | 0.46±0.64 | 26.45±0.28 |
| cIAP-1 | 2.56±1.4 | 21.36±0.07 |
| Claspin | 22.01±1.82 | 7.76±1.25 |
| Clusterin | 15.36±1.85 | 27.69±3.17 |
| HIF-1a | 14.54±3.43 | 9.58±0.86 |
| HO-1/HMOX1/ HSP32 | 7.70±1.05 | 23.74±0.75 |
| HSP70 | 30.23±1.40 | 26.58±1.46 |
| PON2 | 5.46±1.24 | 6.48±0.59 |
| Survivin | 17.20±0.41 | 35.22±4.48 |
| XIAP | 12.06±0.26 | 34.73±0.96 |

**Fig. 5.** Expression levels of anti-apoptotic proteins after 72 h treatment of T-24 cells with *Sp. salmonicolor* $AL_{36}$ extracts obtained from flask or bioreactor culture.

**In conclusion, *Sp. salmonicolor* $AL_{36}$ demonstrates considerably good cell growth in submerged cultivation. The recent study on the dynamics of the fermentation process contributes to the existing knowledge about the biosynthetic potential of these psychrophilic yeasts and points out new possibilities for optimization of the processes. The synthesis of different metabolites depending on the type of cultivation, indicated by the $^1$H NMR fingerprints, and the subsequent difference in the antineoplastic activity of psychrophilic yeast was demonstrated. The active median inhibitory concentrations were mostly below 100 or 50 μg/mL depending on the tumor cell line. The extracts exerted modulating effects on the signal transduction in urinary bladder carcinoma cells.**


## Funding:

**The study was supported by Program for career development of young scientists, Bulgarian Academy of Sciences (Contract № DFNP-60/27.04.2016)**

## Acknowledgements:

**The antiproliferative activity of the extracts on HD-MY-Z, HuT-78 and MJ (MTT-dye assay) was performed on laboratory equipment donated by the Alexander von Humboldt Foundation to Maya M. Zaharieva, PhD in the frame of the program "Equipment subsidies".**