Biotechnological prospecting studies on strains isolated from different natural biotopes in order to obtain biologically active substances and biomaterials

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INTRODUCTION

Enzymes - economic and environmental-friendly biocatalysts → various applications: starch liquefaction, food, paper and textile industries, and pharmaceuticals (***α-amylases***); food and feed (dairy products), detergents, cosmetics and pharmaceuticals, polymer synthesis, agrochemical and environmental fields (***lipases***) (Tomulescu, 2015).

Exopolysaccharides (EPS) - microbial polymers, biodegradable and biocompatible → "*the sleeping giant of biotechnology*" (Tomulescu, 2016).

Antimicrobial natural compounds – antibiotics, biological control (bactericidal, fungicidal effects) (Vladu, 2017).

The main scope of the research: investigation of biotechnological potential of some newly-isolated microorganisms, wild-type strains from various biotopes in Romania.

MATERIALS AND METHODS

Samples of soil, water and vegetal material (pine cones, hay, roots) → microorganisms were deposited in the *CMII-ICCF-WFCC 23 Culture Collection*. Preliminary identification → MALDI-TOF Microflex, 18S rRNA sequencing and BLAST analysis, 16S rDNA - ARDRA technique (Vassu, 2001; Ionescu, 2013).

➢ ***Optimization and kinetics:*** Taguchi L9 and L16 orthogonal arrays, RSM-CCRD and ANOVA; Logistic, Gompertz and Luedeking Piret as kinetic models.

➢ ***Lipase and amylase assays:*** *Willstäter titrimetric method (modified), method with p-nitrophenol laurate as synthetic substrate; Bernfeld method (3,5-dinitrosalicylic acid).*

➢ ***Bioproduct isolation and purification:*** *ultrafiltration and diafiltration (Pellicon module, Merck-Millipore, PLGC/PTGC membranes, 5 and 10 kDa).*

➢ ***Chemical analysis:*** *High Performance Liquid Chromatography, FT-IR and NMR spectroscopic analysis, and Liquid chromatography coupled to UV and electrospray ionization ion trap detection (LC–UV-ESI-MS/MS).*

➢***Pharmacological studies:*** *cytotoxicity assay → the murine fibroblast line L929 (ATCC CRL-6364), Eagle's Minimum Essential Medium; disk diffusion method, Mueller-Hinton, Casein soya-bean digest agar media, test pathogens, Escherichia coli ATCC 8739 and Staphylococcus aureus ATCC 6538.*

MAIN RESULTS

More than 150 microbial strains were isolated from different regions in Romania and screened for enzymes, antibiotics and polysaccharides production.

54 isolates were considered as potential producers of lipolytic enzymes, and 19 strains presented amilolytic activity.

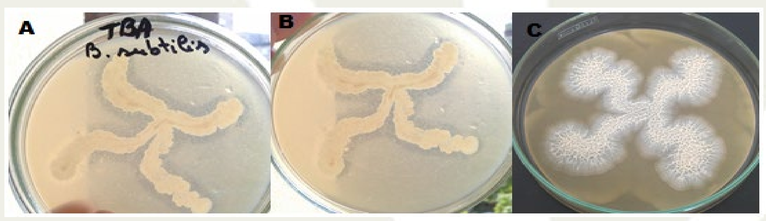


Fig. 1 Lipases/esterases plate assay:
A) and B) *B. subtilis* and C) *Y. lipolytica* on TBA and T80 media

Fig. 2 Amylases - Chapek Dox medium with starch 10 g/L; inhibition zone – antibiotic activity of *B. subtilis* against *S. aureus* ATCC 6538

Polysaccharide from the *Klebsiella oxytoca* ICCF 419 high level of cytoprotection → biomaterial pharmaceutical applications → healing effect of the wounds → medical devices → scarring plasters.

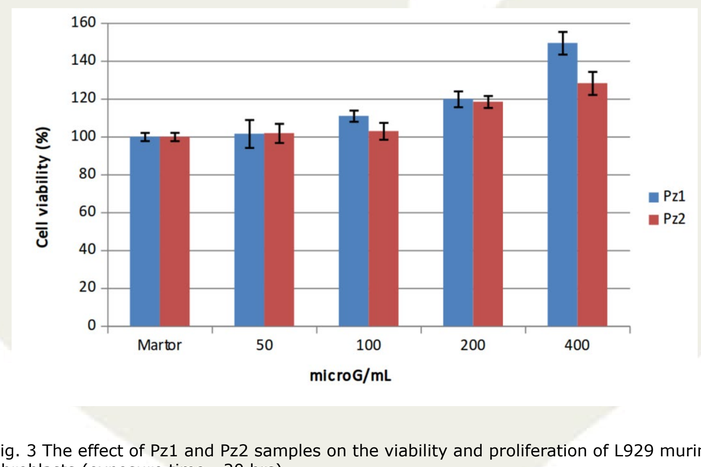


Fig. 3 The effect of Pz1 and Pz2 samples on the viability and proliferation of L929 murine fibroblasts (exposure time - 20 hrs)

➢ ***Klebsiella oxytoca ICCF 419:***

- **2.77 g/L purified polysaccharide**;
- possible a novel chemical structure?: glucose, mannose, glucuronic acid, galactose, and fucose are the main EPS components according to the carbohydrate fingerprint.

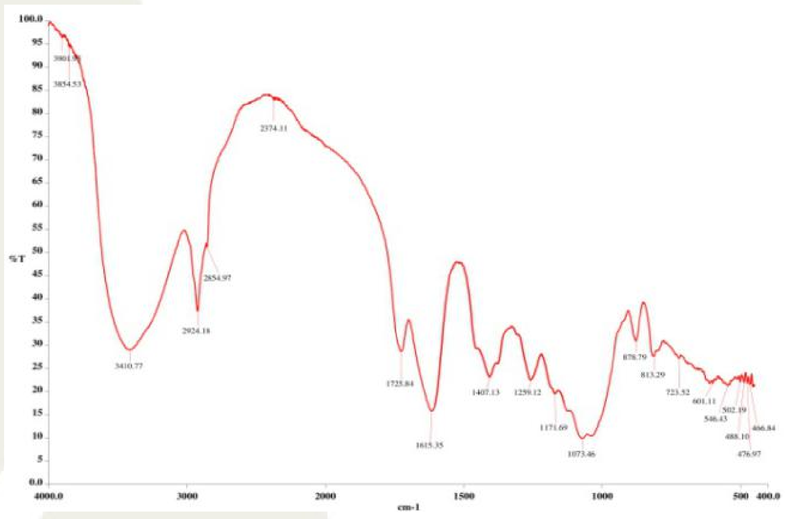


Fig. 4 FTIR spectrum of the purified exopolysaccharide

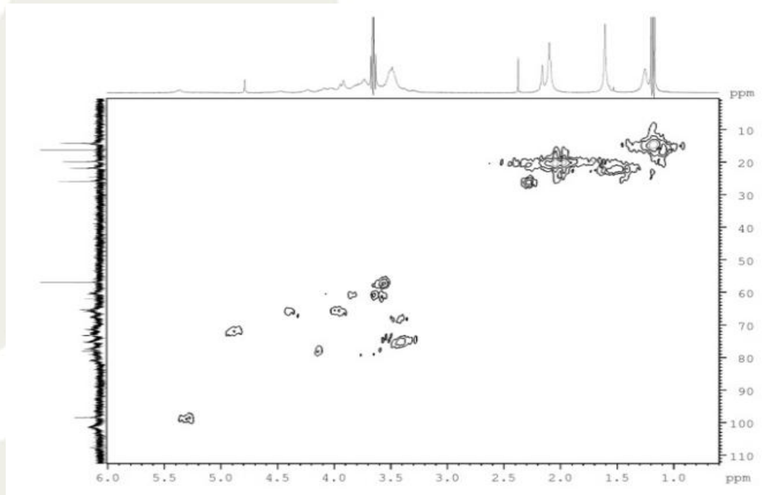
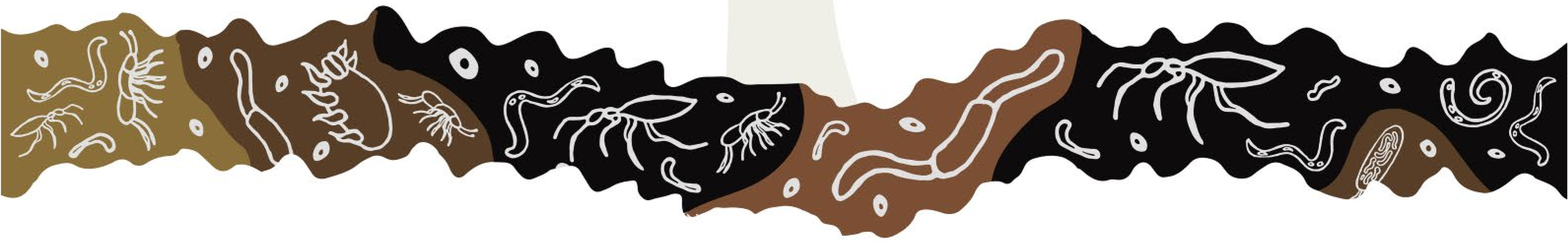


Fig. 5 2D HMQC-NMR spectrum of the exopolysaccharide

➢**Enzymes:**

▪**Lipase**: ***Galactomyces geotrichum*** **ICCF 415** – 39.3 UL FIP/mL (Taguchi L9 optimization), 36 hrs fermentation; 27 U/mL - p-nitrophenol laurate as synthetic substrate, 60 hrs fermentation.

▪**Amylase:** ***Bacillus mycoides*** - 10.87 U/mL, 48 hrs fermentation; electrophoretic profile → 60 kDa.



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