# DRAFT GENOME OF A USEFUL BACTERIA: *PAENARTHROBACTER NICOTINOVORANS*

Iasmina Honceriu, Razvan Stefan Boiangiu, Marius Mihasan
BioActive research group, Faculty of Biology, "Alexandru Ioan Cuza" University of Iasi, Iasi, Romania

## 1. Introduction

*P. nicotinovorans* is a Gram-positive soil bacteria with the ability of nicotine degradation due to the pAO1 megaplasmid (**Fig. 1; Fig. 2**). Thus, it can have applications in the bioremediation process and the pharmaceutical industry e.g. 6-hydroxy-nicotine (**Fig. 3**)

**Fig. 1**: The Nicotine-Blue (NB) metabolite accumulating in the growth medium

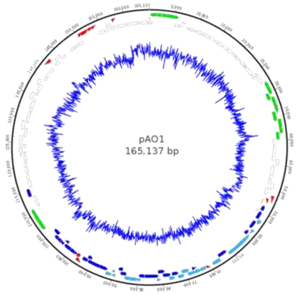


**Fig. 2**: The pAO1 catabolic megaplasmid (165k bp), including the *nic*-genes cluster

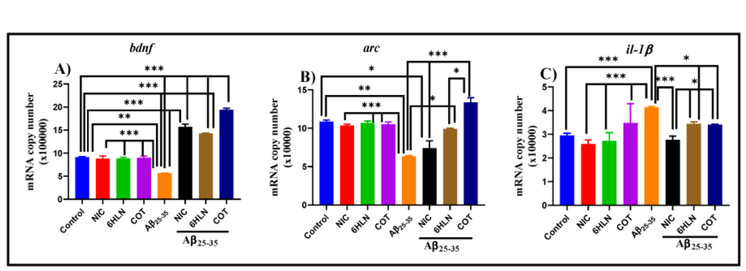


**Fig. 3**: 6-hydroxy-nicotine has a neuroprotective effect in the hippocampus of laboratory rats
https://doi.org/10.3390/antiox9080768

## 2. Sequencing and assembly

**2.1. gDNA isolation (Fig. 4)** –DNeasy UltraClean Microbial Kit, Qiagen

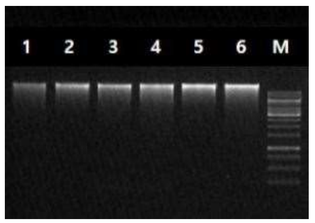


**Fig. 4**: 1, 2, 3, 4, 5, 6 - Isolated *P. nicotinovorans* gDNA
1% agarose gel, 30min running at 100V, 0.5ul of DNA loaded

**2.2. Sequencing** – Illumina Platform, Paired-end, TrueSeq PCR Free (done by Macrogen) **(Fig. 5)**


**Fig. 5**: Sequencing steps

**2.3. Data analysis**

**Raw data quality check: fastQC v. 0.11.9**

**Trimming, clipping and filtering: fastp**

***De novo* assembly: SPAdes v. 3.15.2**

**Estimated completeness and contamination: CheckM 1.1.3 and QUAST v. 5.0.2**

**Gene finding and annotation: RAST**

## 3. Assembled genome

Paenarthrobacter nicotinovorans pAO1+

| Check M | | Quast | |
|---|---|---|---|
| GC | 0,6315665 | GC (%) | 63,16 |
| GC std | 0,01667738 | | |
| Genome size | 4443122 | Total length | 4443122 |
| #ambigous bases | 0 | # N's per 100 kbp | 0 |
| #contigs | 31 | # contigs | 31 |
| Longest contig | 778056 | Largest contig | 778056 |
| Mean contig length | 143326,516 | | |
| N50 (contigs) | 270987 | N50 | 270987 |
| | | N75 | 175114 |
| | | L50 | 4 |
| | | L75 | 9 |
| Coding density | 0,90563235 | #predicted genes (unique) | |
| Translation table | 11 | # contigs (>= 0 bp) | 31 |
| #predicted genes | 4132 | # contigs (>= 1000 bp) | 31 |
| Completeness | 99,7076023 | # contigs (>= 5000 bp) | 26 |
| Contamination | 0 | # contigs (>= 10000 bp) | 24 |
| | | # contigs (>= 25000 bp) | 22 |
| | | # contigs (>= 50000 bp) | 18 |
| | | Total length (>= 0 bp) | 4443122 |
| | | Total length (>= 1000 bp) | 4443122 |
| | | Total length (>= 5000 bp) | 4431906 |
| | | Total length (>= 10000 bp) | 4417073 |
| | | Total length (>= 25000 bp) | 4388354 |
| | | Total length (>= 50000 bp) | 4234855 |

Paenarthrobacter nicotinovorans pAO1-

| Check M | | Quast | |
|---|---|---|---|
| GC | 0,63289135 | GC (%) | 63,29 |
| GC std | 0,02016193 | | |
| Genome size | 4276881 | Total length | 4276881 |
| #ambigous bases | 0 | # N's per 100 kbp | 0 |
| #contigs | 30 | # contigs | 30 |
| Longest contig | 867056 | Largest contig | 867056 |
| Mean contig length | 142562,7 | | |
| N50 (contigs) | 650853 | N50 | 650853 |
| | | N75 | 203532 |
| | | L50 | 3 |
| | | L75 | 8 |
| Coding density | 0,90922637 | #predicted genes (unique) | |
| Translation table | 11 | # contigs (>= 0 bp) | 30 |
| #predicted genes | | # contigs (>= 1000 bp) | 30 |
| Completeness | 99,7076023 | # contigs (>= 5000 bp) | 22 |
| Contamination | 0 | # contigs (>= 10000 bp) | 21 |
| | | # contigs (>= 25000 bp) | 19 |
| | | # contigs (>= 50000 bp) | 16 |
| | | Total length (>= 0 bp) | 4276881 |
| | | Total length (>= 1000 bp) | 4276881 |
| | | Total length (>= 5000 bp) | 4260736 |
| | | Total length (>= 10000 bp) | 4250956 |
| | | Total length (>= 25000 bp) | 4207876 |
| | | Total length (>= 50000 bp) | 4098091 |

## 4. Conclusions

The draft genome of *Paenarthrobacter nicotinovorans* strain pAO1+ is 4.4 Mbp in size and consists of 31 contigs with an N50 of 270 kbp.
The draft genome of pAO1- is 4.2 Mbp in size and consists of 30 contigs, with an N50 of 650 kbp.
The difference between the two genomes is one contig of approx. 165 kbp that corresponds to the pAO1 plasmid.

This work was supported by PNIII-P4-ID-PCE-2020-0656 research project financed by UEFISCDI, Romania.

# THE STIMULATORY EFFECT OF THE ROMANIAN MONOFLORAL HONEY ON KERATINOCYTE PROLIFERATION

Andreea IOSAGEANU [1,2], Elena MIHAI [2], Oana CRACIUNESCU [2], Anisoara CIMPEAN [1]

[1] University of Bucharest, Faculty of Biology, Splaiul Independentei 91-95, Bucharest, R-050095, Romania
[2] National Institute of R&D for Biological Sciences, 296, Splaiul Independentei, 060031 Bucharest, Romania
E-mail: andreea.iosageanu@gmail.com

## Introduction

Clinical studies have reported the successful use of honey in skin wound treatment. However, more complex studies are required to identify and understand how certain compounds act on the wound healing process.

**The aim of this study** was to evaluate the effect of different honey varieties on keratinocyte proliferation *in vitro* and to correlate their biological activity to the physicochemical characteristics.

## Methods

Three honey samples of different floral origin were analysed in this study: **linden**, **sunflower** and **rapeseed honey**. For the melissopalynological analysis, we have used the nonacetolytic method. For the physicochemical characterization, the moisture, pH and free acidity were analysed. The total phenolic content (**TPC**) was determined by Folin-Ciocâlteu method and the ascorbic acid (**AA**) content by the DCPIP method. The effect of honey samples on HaCaT keratinocyte proliferation was evaluated by the Neutral Red assay after 24 and 48 h of incubation, in comparison to untreated control. Cellular morphology observation was performed in a similar experiment using phase-contrast microscopy.

## Results

**Table 1**. Physicochemical characterization of honey samples.

| Honey sample | Pollen types | Moisture | pH | F. acidity | TPC | AA |
|---|---|---|---|---|---|---|
| Linden honey | *T. platyphyllos* 56%, *H. annuus* 20% | 16.4 | 4 | 5.4 | 450 | nd |
| Sunflower honey | *H. annuus* 54%, *B. napus* 21%, *T. platyphyllos* 12% | 18.6 | 3.8 | 7.8 | 560 | 2.5 |
| Rapeseed honey | *B. napus* 63%, *H. annuus* 25% | 18.8 | 3.9 | 6.3 | 510 | nd |

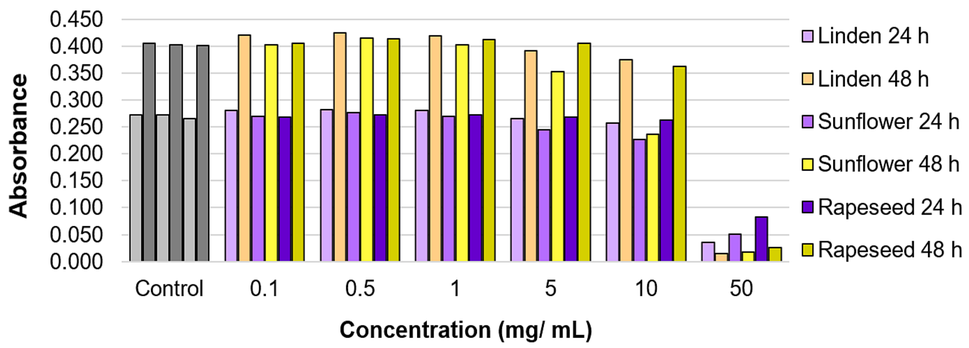


**Figure 1**. Effect of honey samples on HaCaT cell proliferation after 24 and 48 h of incubation, determined by Neutral Red assay. Cell viability tests indicated a wide range of biocompatibility (0.1-5 mg/mL) of all honey samples.

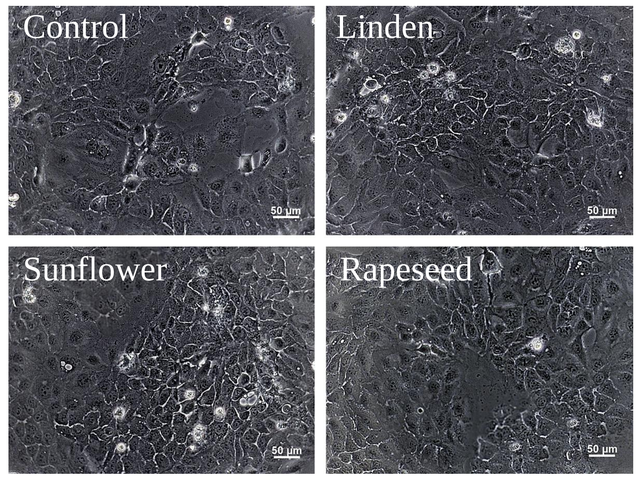


**Figure 2**. Cell morphology of HaCaT cells treated with 0.5 mg/ mL honey for 48 h.

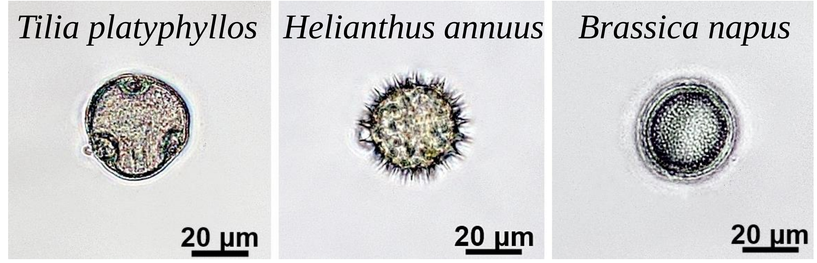


**Figure 3**. Dominant pollen types found in honey samples.

## Conclusions

The physicochemical characterization proved the quality of honey. TPC values ranged from 450 mg GAE/kg honey for linden honey to 560 mg GAE/kg honey for sunflower honey. The ascorbic acid content was 2.5 mg/kg honey for sunflower honey and undetectable for the other two samples (**Table 1**). A stimulation of keratinocyte proliferation was observed in case of all honey samples by up to 5% (**Figure 1**). No alterations in cell morphology were observed (**Figure 2**). The pollen analysis confirmed the botanical origin of honey samples (**Figure 3**). Based on these promising results we envision further studies to test the honey potential for wound healing applications.

# Organic acids production by phosphate solubilizing bacteria

Mantea Loredana-Elena, Mihășan Marius, Ștefan Marius

Faculty of Biology, , "Alexandru Ioan Cuza" University of Iași, Iași Romania

## Introduction

Microorganisms are involved in a range of processes that affect the transformation of soil phosphorus. Phosphate solubilizing bacteria (PSB) have the ability to convert insoluble phosphorus compounds in soluble forms using different mechanisms. It is generally accepted that the main mechanism of mineral phosphate solubilization by PSB strains is the decrease of soil pH due to the release of low molecular weight organic acids.Therefore, the main goal of this study was to identify the organic acids involved in tricalcium phosphate solubilization for three bacterial strains (P1.5S, P3.4S and D12).

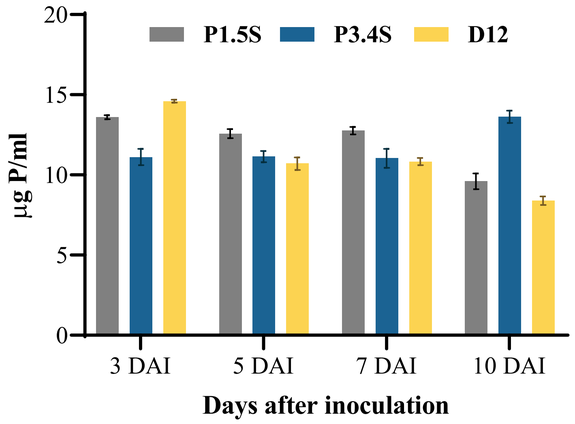


**Fig.1. Tricalcium phosphate solubilization by the bacterial strains**

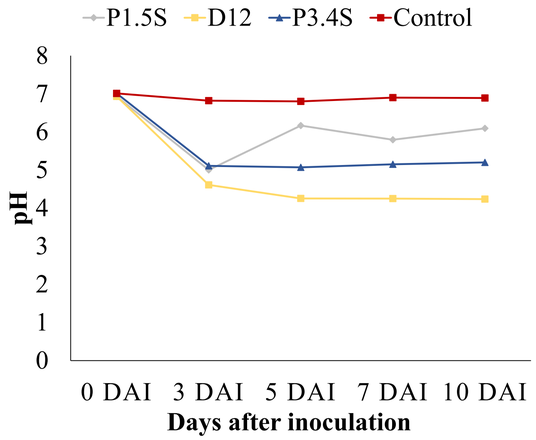


**Fig.2. pH variation during ten days after inoculation**

## Methods

The bacterial strains used in this study was isolated fron soils cultivated with maize (P1.5S and P3.4S) and municipal wastewater. The ability of the bacterial strains to solubilize tricalcium phosphate was determined at 28°C using phosphomolybdate ammonium method. Also, the pH of the bacterial cultures was measured and the organic acids were identified using HPLC (High Performance Liquid Chromatography)

## Results

**Quantitative screening of isolated bacterial strains**. The amounts of solubilized phosphorus by the tested bacterial strains ranged between 8,36 and 14,59 µg/mL, the highest amount being recorded in the case of D12 strain at 3 DAI (days after inoculation) (fig.1).

**Mechanism involved in tricalcium phosphate solubilization**. The bacterial strains caused a decrease in the pH of the medium (fig 2) due to the production of organic acids: malic/formic, tartaric, lactic, acetic, citric, succinic and oxalic. All the tested bacterial strains produced malic/formic acid, the highest concentration being recorded in the case of P3.4S strain (25,84 mM) (fig. 4). Succinic acid was produced in a higher concentration by P1.5S strain (6,37 mM) (fig.3), in contrast to P3.4S strain where the maximum concentration was 2,14 mM (fig. 4). Regarding D12 strain, there is no significant difference between the concentrations of lactic and acetic acids at 3, 5 and 10 DAI. The same was observed for tartaric and malic/formic acids at 5, 7 and 10 DAI (fig. 5).

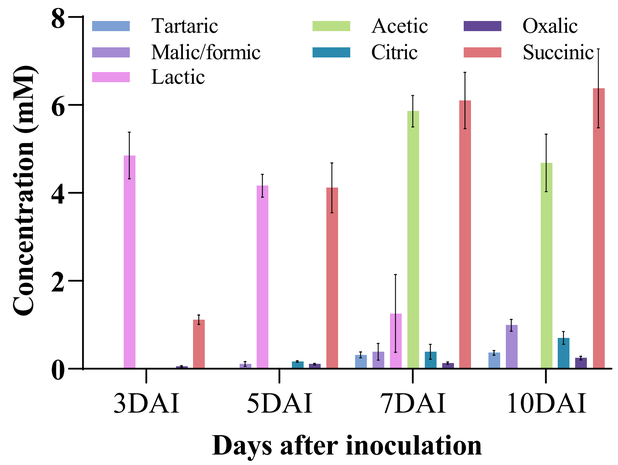


**Fig.3. Organic acids produced by P1.5S strain**

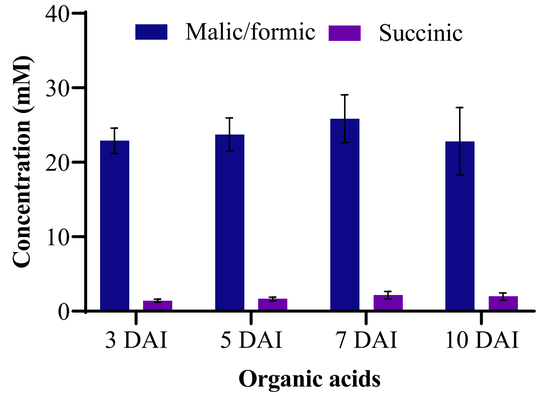


**Fig.4. Organics acids produced by P3.4S strain.**

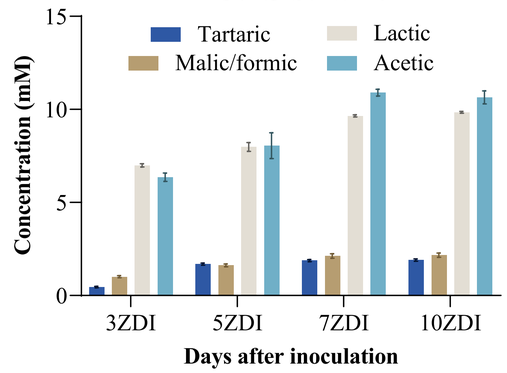


**Fig.5. Organic acids produced by D12 strain**

## Conclusions

One of the mechanisms involved in tricalcium phosphate solubilization by the tested bacterial strains is the acidification of the culture medium due to the production of organic acids.

# Identification of invertebrates using DNA barcoding. Study case: Danube River – Danube Delta – Black Sea System

Menabit Selma[1 2], Begun Tatiana[1], Teaca Adrian[1], Muresan Mihaela[1], Pavel Ana Bianca[1], Iancu Lavinia[2], Purcarea Cristina[2]

[1]National Institute of Marine Geology and Geo-Ecology (GeoEcoMar), Bucharest, Romania, [2]Institute of Biology Bucharest, Department of Microbiology, Bucharest, Romania

## Introduction

The study was of major interest due to the lack of molecular data on the investigated groups from the focused area, which is shelter to many endemic and Ponto-Caspian species. Therefore, we aimed to identify marine and freshwater invertebrates using DNA barcoding based on cytochrome c oxidase subunit 1 gene sequencing.

## Methods

Samples were collected in 2019 and 2020, by using two different Van Veen grabs (0.135 $m^2$; 0.04 $m^2$) (Figure 1). Each specimen selected for the genetic analysis was washed with sterile water and preserved in 200 µl Tris-EDTA pH 8 buffer at -20° C. Fragments of mitochondrial gene COI were PCR amplified using universal forward (LCO1490: 5'-GGTCAACAAATCAAA-GATATTGG-3') and reverse (HCO2198: 5'-T.AAACTTCAGGGTGAC-CAAAAAATCA-3') primers (Folmer et al., 1994).

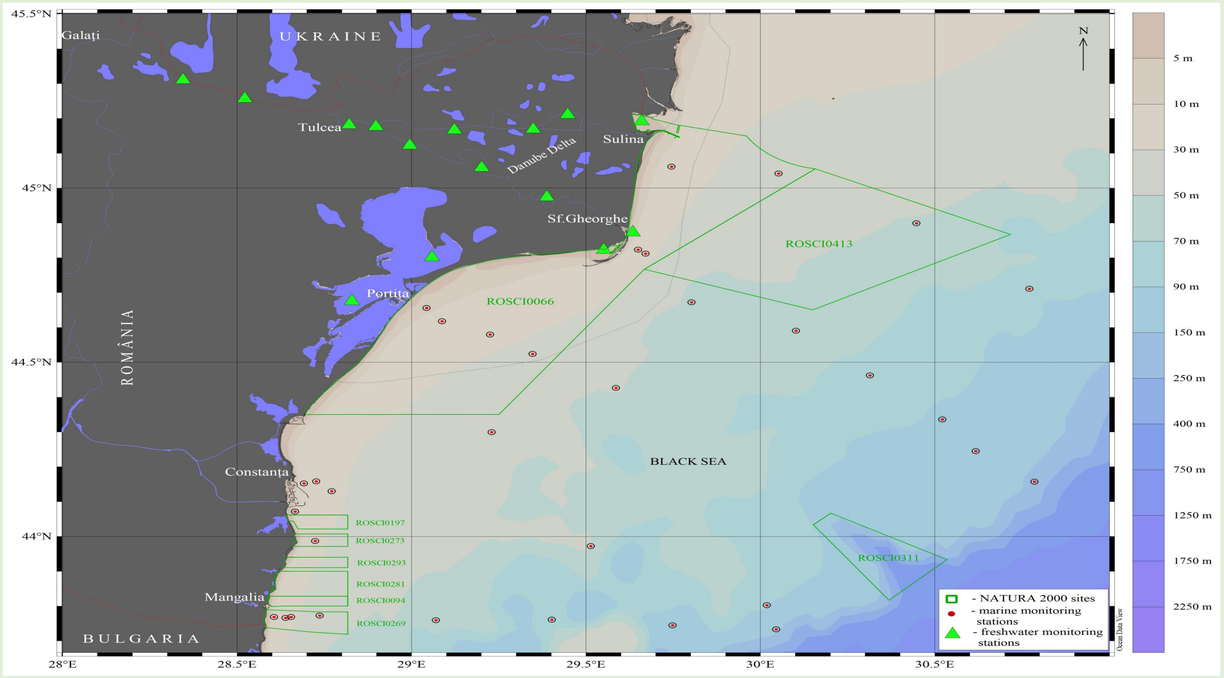


**Figure 1. Map with the study area**

## Results

To validate the morphological identification through DNA barcoding, a similarity of 97% or higher was used as threshold for the species-level matching (Hebert et al. 2003). All individuals were identified at species level, showing a match of at least 97% with the homologous COI gene sequences. The identified invertebrate COI sequences were deposited in GenBank (Table 1).

**Table 1. Best match COI gene sequence and GenBank acc. nr. of invertebrate species**

| Crt. nr. | GenBank Species | Cover/Identity | GenBank Coresp. Seq. | Acc. Nr. GenBank |
|---|---|---|---|---|
| 1. | *Halisarca cf. dujardini* Johnston, 1842 | 100%/97.77% | EU237483.1 | MW139668 |
| 2. | *Erpobdella octoculata* Linnaeus 1758 | 98%/97.85% | MT410851.1 | MW600087 |
| 3. | *Helobdella stagnalis* Linnaeus, 1758 | 100%/99.85% | MF150165.1 | OK559910 |
| 4. | *Physa fontinalis* Linnaeus, 1758 | 98%/99.42 % | FJ373018.1 | MW600069 |
| 5. | *Radix lagotis* Schrank, 1803 | 97%/ 99.27 % | LT623602.1 | MW600073 |
| 6. | *Bithynia tentaculata* Linnaeus 1758 | 97%/99.20% | MK308219.1 | MW139681 |
| 7. | *Microcolpia daudebartii acicularis* Ferussac, 1823 | 99%/99.37% | FJ652188.1 | MW139680 |
| 8. | *Theodoxus transversalis* C. Pfeiffer, 1828 | 100%/100% | HM171591.1 | MW139678 |
| 9. | *Abra alba* W. Wood, 1802 | 97%/87.93% | KR084667.1 | MW139667 |
| 10. | *Mya arenaria* Linnaeus, 1758 | 99%/99.69% | KF360141.1 | MW600092 |
| 11. | *Papillicardium papillosum* Poli, 1791 | 100%/97.44% | EU733111.1 | MW139666 |
| 12. | *Dikerogammarus haemobaphes* Eichwald, 1841 | 100%/99.84% | MN343237.1 | MW600089 |
| 13. | *Dikerogammarus villosus* Sowinsky, 1894 | 97%/99.53% | MK159942.1 | MW600090 |
| 14. | *Chelicorophium robustum* G.O. Sars, 1895 | 97%/99.64% | KM009061.1 | MW139683 |
| 15. | *Limnomysis benedeni* Czerniavsky, 1882 | 97 %/99.51% | AY529021.1 | MW600070 |
| 16. | *Paramysis lacustris* Czerniavsky, 1882 | 97%/99.47% | KT193643.1 | MW600085 |
| 17. | *Asellus aquaticus* Linnaeus, 1758 | 98%/100% | KJ676764.1 | MW139672 |
| 18. | *Caenis robusta* Eaton, 1884 | 100%/98.79% | MT628575.1 | MW600086 |
| 19. | *Neureclipsis bimaculata* Linnaeus, 1758 | 100%/100% | KJ675356.1 | MW600076 |
| 20. | *Leptocerus tineiformis* Curtis, 1834 | 100%/99.53% | MT584164.1 | MW600088 |
| 21. | *Molgula manhattensis* De Kay, 1843 | 100% /97.80% | AB922015.1 | OK272500 |

## Conclusions

The molecular method proved to be successful for all the analysed individuals, being very helpful to assign the exact taxon to those misidentified through morphotaxonomical criteria. Our results suggest that DNA barcoding represent a valuable and efficient tool for species discrimination. However, combining phenotypic and molecular criteria increase the accuracy and reliability of results.

**References**

O. Folmer, M. Black, W. Hoeh, R. Lutz, R. Vrijenhoek, DNA primers for amplification of mithocondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates, Mol. Mar. Biol. Biotechnol. 3 (1994) 294-297;

P.D.W. Hebert, A. Cywinska, Sh.L. Ball, J.R. deWaard, Biological identification through DNA barcode, Proc. R. Soc. Lond. B. (2003) 313-321.

# Antibiotic resistance of *Paenarthrobacter nicotinovorans* – an *in-silico* and *in-vitro* study

**Andreea-Mihaela Mlesniță[1], Veronica-Cristina Moldovan[1], Fakhri Kallabi1[1,2], Marius Ștefan[1], Marius Mihășan[1]**

[1] BioActive Research Group, Faculty of Biology, "Alexandru Ioan Cuza" University of Iași, Iași Romania, [2] University of Sfax, Faculty of Medicine of SFAX, Sfax, Tunisia.

## Introduction

The ability to metabolize nicotine is one of the most useful traits of *Paenarthrobacter nicotinovorans* pAO1 strain. Encoded by the pAO1 megaplasmid (Fig. 1), the catabolic pathway converts nicotine to a blue pigment (Fig. 2) and can be used to produce useful chemicals.

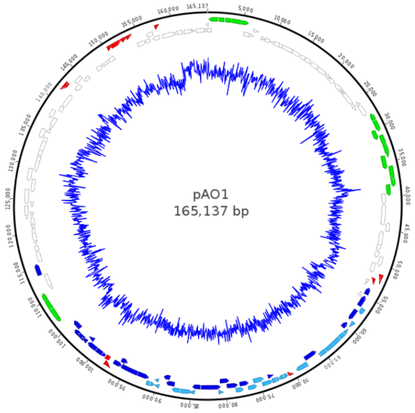


**Fig. 1**. *Paenarthrobacter nicotinovorans* pAO1 megaplasmid

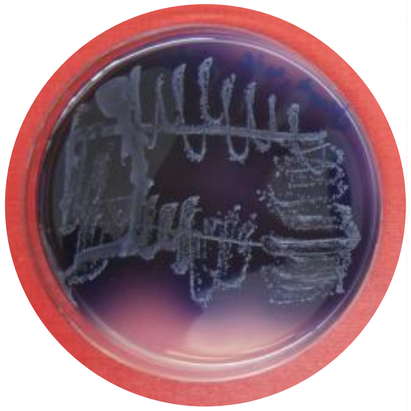

**Fig. 2**. The Nicotine-Blue (NB) metabolite accumulating in the growth medium

## Methods

- draft genome NGS sequencing - Illumina NovaSeq 6000;
- genome assembly - SPAdes 3.14.1;
- *in-silico* resistance genes detection - CARD;
- *in-vitro* resistance testing - Kirby-Bauer method, diameters of inhibition referenced to CLSI's Performance Standard for Antimicrobial Susceptibility Testing.

## Results

An overview of the 255 CARD loose hits is presented in Fig. 3

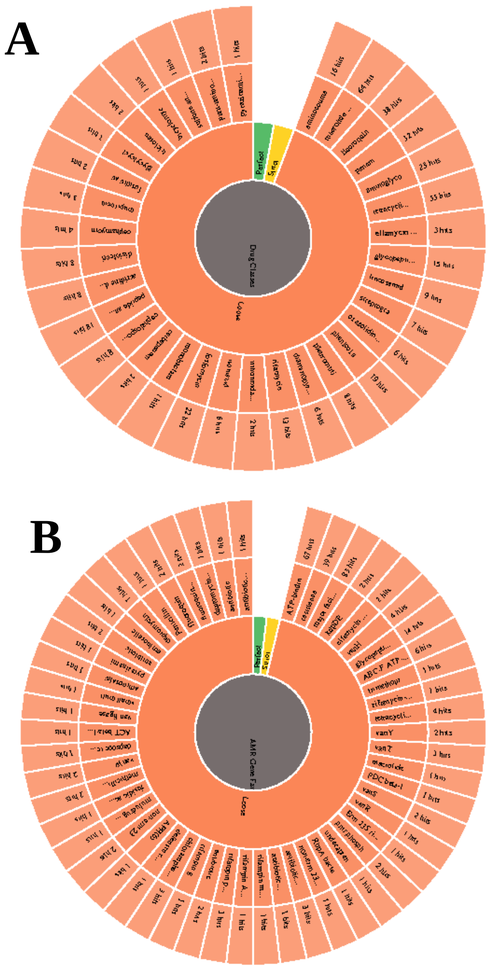


**Fig. 3**. Loose hits identified by CARD - A. AMR gene family; B - Drug Class

| Antibiotic class | Best Identities | Percentage Length | Antibiotic | Antibiogram result |
|---|---|---|---|---|
| **Aminoglycoside** | 51.33 | 99.56 | Spectinomycin | R on MHA & C+N, S on C |
| | | | Gentamicin | R on C+N, S on MHA & C |
| | | | Kanamycin | MS on C, S on MHA & C+N |
| | | | Neomycin | R on C+N, S on MHA & C |
| **Glycopeptide** | 58.82 | 95.28 | Vancomycin | R on C+N, S on MHA & C |
| **Macrolide antibiotic** | 55.76 | 80.00 | Erithromycin | R on C+N, MS on C, S on MHA |
| **Macrolide antibiotic, penams** | 73.54 | 98.25 | | |
| **Cephalosporins** | 57.43 | 96.79 | Ceftriaxone | R on all mediums |

**Table 1.** CARD hits validated by *in-vitro* experiments

The hits were filtered to 14 major hits based on >50% aac identity with a known AMR gene family. A total of 15 antibiotics comprising 7 of the CARD major hits and 2 non-hits were tested in the lab.

*P. nicotinovorans* was found to be resistant to 7 antibiotics corresponding to 5 CARD major hits, as seen in table 1. One interesting find is the difference of the antibiogram results depending on the growth medium (Fig. 4).

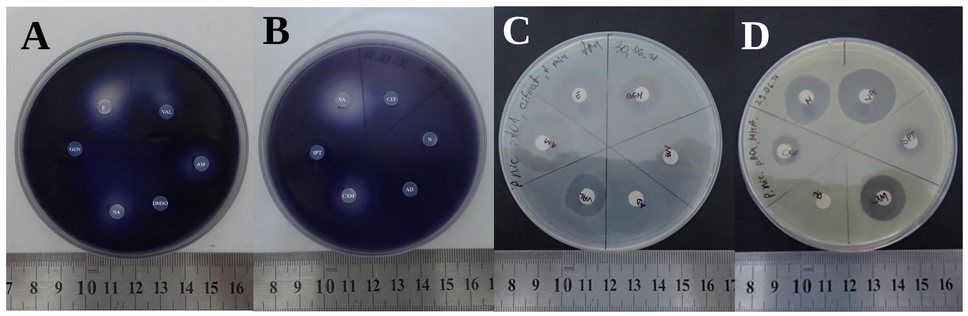


**Fig. 4**. Test plate examples showing antibiotic resistance of *P. nicotinovorans* when grown on nicotine, citrate and MHA media.

## Conclusions

The antibiotic resistance profile of the strain has been established. The sensibility of *P. nicotinovorans* to certain antibiotics is modulated by the presence of nicotine in the growth medium.

# Study of the mechanisms of antibiotic resistance of bacteria isolated from the influent of a wastewater treatment plant

Moldovan Cristina-Veronica, Ștefan Marius
Faculty of Biology, , "Alexandru Ioan Cuza" University of Iași, Iași ,Romania

## Introduction

Wastewater treatment plants (WWTP) are important hotspots for the selection of antibiotic resistant bacteria and antibiotic resistance genes. These plants collect wastewater from different sources polluted with various antimicrobial substances that drives the selection of resistant microorganisms. The reuse of wastewater after treatment process leads to the dissemination of clinically important bacteria and resistance genes along surface waters and soil. The goal of our study was to investigate the spread of resistant bacteria in the influent of an urban WWTP and to analyze the resistance mechanism of the tested bacteria.

## Methods

***Isolation of resistant bacteria*** was performed by serial dilution technique, using culture media supplemented with highly used antibiotics in therapy (Esiobu et al., 2002).

***Antimicrobial susceptibility testing*** was evaluated using minimum inhibitory concentration (MIC) assay (Elshikh et al., 2016).

***Detection of carbapenemase-producing Enterobacteriaceae*** was determined using *Brilliance CRE Agar* (Bialvaei et al., 2016).

***Biofilm formation*** was assessed using crystal violet method (Stepanović et al., 2007).

## Results

### *Resistant bacteria*

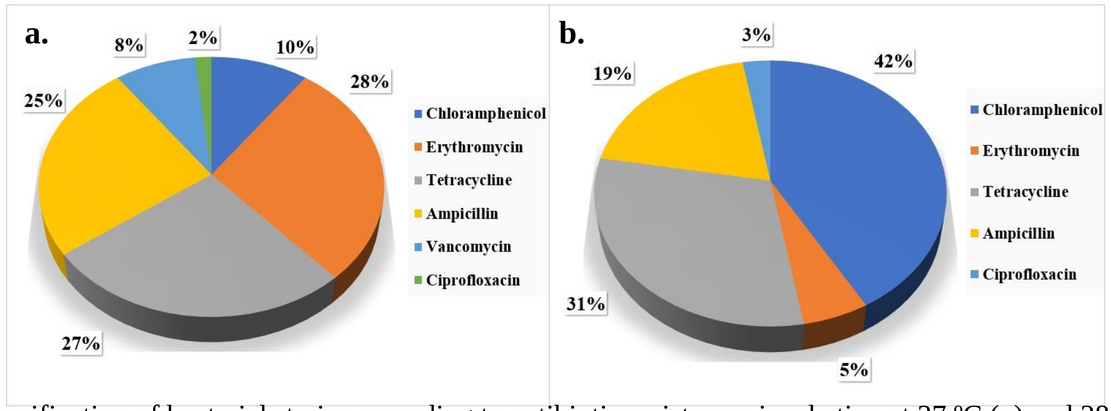


**Fig.1**. Classification of bacterial strains according to antibiotic resistance: incubation at 37 ºC (**a**) and 28 ºC (**b**).

### *Minimum inhibitory concentration*

**Tab. 1**. Highest MICs values of antibiotics for resistant strains

| MIC (µg/ml) | | | | |
|---|---|---|---|---|
| **Ampicillin** | **Tetracycline** | **Chloramphenicol** | **Erythromycin** | **Vancomycin** |
| ≥ 4096 | 256 | ≥ 1024 | ≥ 1024 | 512 |

### *Carbapenemase-production organisms*

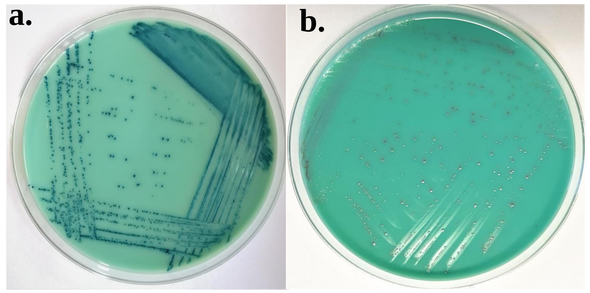


**Fig. 2**. Different resistant strains on *Brilliance CRE Agar*: **a.** *Klebsiella* – blue; **b.** *E. coli* – light pink

**Blue colonies – 9 strains**
(*Klebsiella/Enterobacter/Serratia/Citrobacter)*

**Light pink colonies - 4 strains**
(*E. coli*)

**Cream colonies / White – 21 strains**
(*Acinetobacter)*

### *Biofilm production*

**Tab. 2**. Bacterial cell adherence during 24h

| Strains | 24 hours | | |
|---|---|---|---|
| | **OD** | **ODc** | **Adherence** |
| $V_{37}A_1$ | 0.334 | 0.086 | ++ |
| $V_{37}A_2$ | 0.246 | 0.086 | ++ |
| $V_{37}A_3$ | 0.156 | 0.086 | + |
| $V_{37}Cl_1$ | 0.032 | 0.086 | - |
| $V_{37}T_1$ | 0.222 | 0.086 | ++ |
| $V_{37}T_2$ | 0.164 | 0.086 | + |
| $V_{37}T_{11}$ | 0.116 | 0.086 | + |
| $V_{37}T_{12}$ | 0.068 | 0.086 | - |
| $V_{37}E_1$ | 0.148 | 0.086 | + |
| $V_{37}E_3$ | 0.098 | 0.086 | + |
| $V_{37}E_4$ | 0.082 | 0.086 | - |

- = no biofilm producer
+ = weak biofilm producer
++ = moderate biofilm producer

OD = optical density
ODc = cut-off value

## Conclusions

- **96 resistant strains** were isolated during performed experiments.
- 55.96% of Gram-negative bacterial strains were **carbapenemase-producing organisms**.
- Among the tested strains, 3 formed a **moderate adherent biofilm**, while 5 formed a **weakly adherent biofilm**, which is an intrinsic resistance mechanism to antibiotics.
- These results suggest that the investigated strains present both acquired and intrinsic mechanisms of resistance.

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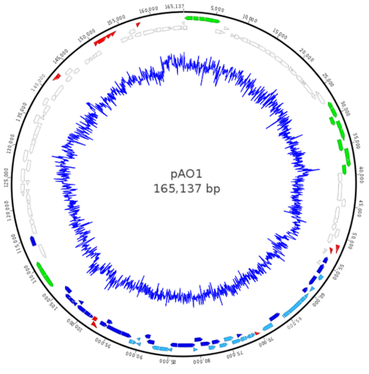
4. Stepanović et al., 2007, *APMIS*, **115**: 891-899.

# CRISPR-Cpf1 system and its utility in editing the *Paenarthrobacter nicotinovorans* genome

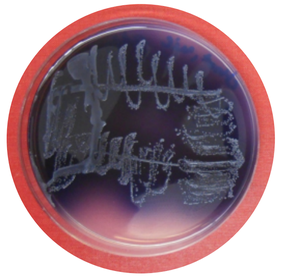
Iustin-Tiberius Munteanu[1], Fakhri Kallabi[1] and Marius Mihăşan[1]

[1]*BioActive Research Group, Faculty of Biology, Alexandru Ioan Cuza University of Iași, Romania*

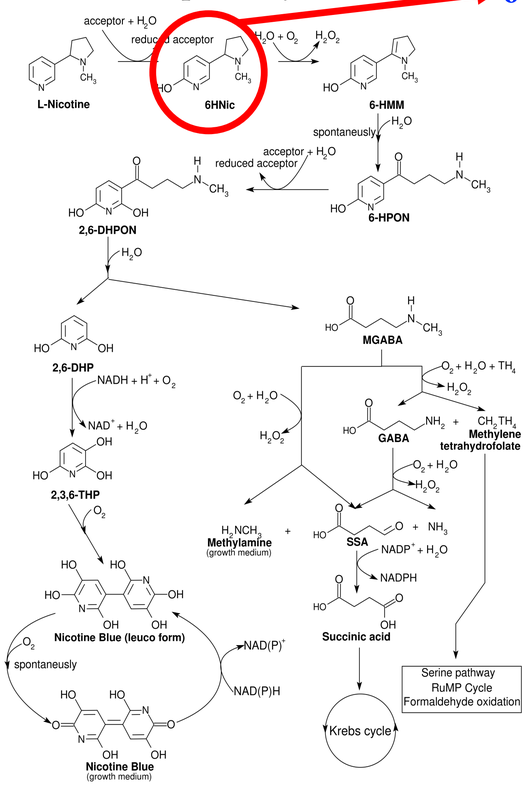
## 1. Nicotine degradation pathway of *Paenarthrobacter nicotiovorans*




pAO1 catabolic megaplasmid encoding the nicotine pathway

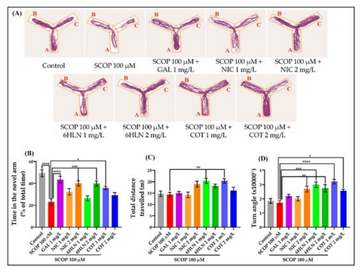


The Nicotine-Blue (NB) metabolite accumulating in the growth medium



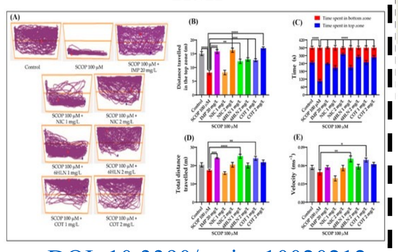

## 6-hydroxy-L-nicotine

Improves memory ....



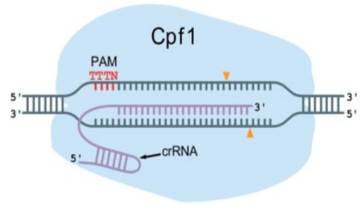
DOI: 10.3390/antiox10020212

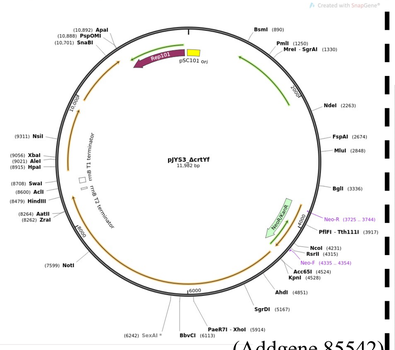
... And reduces anxiety like behavior in zebra-fish



DOI: 10.3390/antiox10020212

## 2. CRISPR system: from the immune system to genome editing




(Addgene 85542)

KUNGL. VETENSKAPS AKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES

The Nobel Prize in Chemistry 2020
Emmanuelle Charpentier
Jennifer A. Doudna

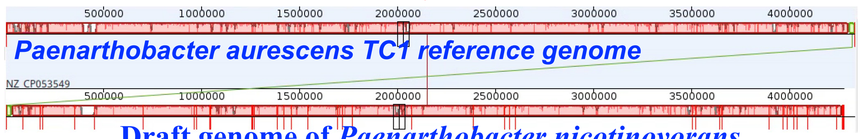
Press release: The Nobel Prize in Chemistry 2020

## 3. Check the presence of CRISPR systems in *Paenarthrobacter nicotinovorans*

UNIVERSITÉ PARIS-SUD 11

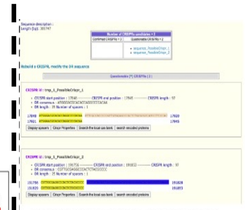
**CRISPRs web server**
(https://crispr.i2bc.paris-saclay.fr/)

I2BC

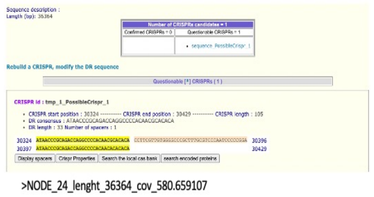



**Draft genome of *Paenarthrobacter nicotinovorans***

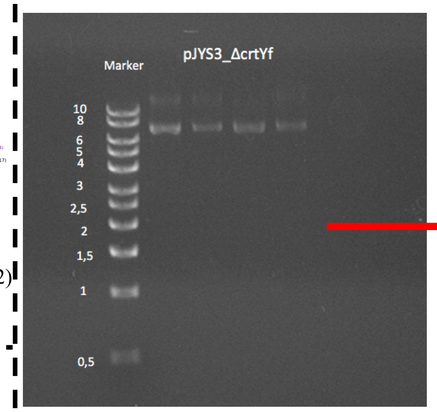
(BioProject accession PRJNA693273)



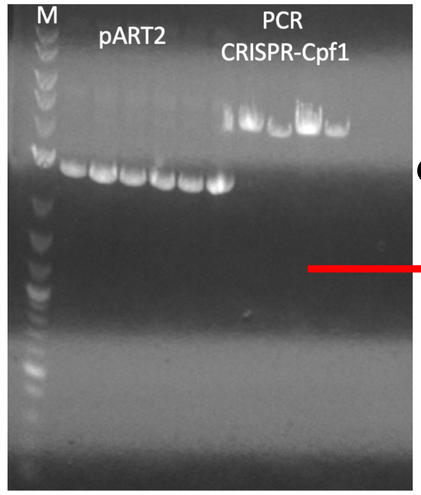



## 4. Test CRISPR-Cpf1 system into *Paenarthrobacter nicotinovorans*




No transformants upon selection with kanamycin




Clone the CRISPR genes into a plasmid known to work in *Paenarthrobacter nicotinovorans*- pART2

References:

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# Anti-*Candida* activity of synthetic flavonoid Br-Cl

Savu Mihaela[1], Bîrsa Lucian[2], Ștefan Marius[1]

[1]Faculty of Biology, "Alexandru Ioan Cuza" University of Iași, Iași Romania
[2]Faculty of Chemistry, "Alexandru Ioan Cuza" University of Iași, Iași Romania

## Introduction

Antibiotic resistance is one of the biggest threats to global health, food security, and development today. Therefore, the discovery of new drugs with high efficacity and low toxicity is a priority in a scientific reports. A potential solution could be represented by synthetic flavonoids due to their important antimicrobial activity. In this context, we investigated anti-*Candida* activity of a new synthetic flavonoid with bromide as halogen substituent at the benzopyran core (Br-Cl).

**Table 1** – The MICs value of flavonoid Br-Cl against *Candida* sp.

| ***Candida* sp. strains** | **MIC (µg/mL)** |
|---|---|
| *Candida albicans* Prx | 15.62 |
| *Candida krusei* | 15.62 |

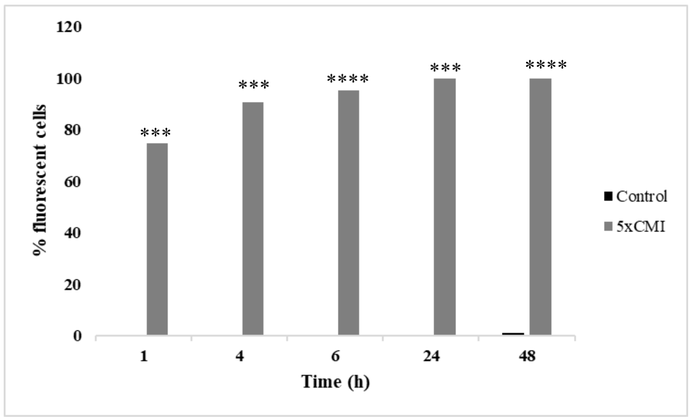



**Figure 1** – Effect of Br-Cl flavonoid on *C. albicans* membrane structure *** = P < 0.0008; **** = P < 0.0001.

## Conclusion

- Our results showed that flavonoid Br-Cl has a high potential to be use as an efficient antifungal agent.

**Acknowledgements**

This work was supported by a grant of the Romanian Ministry of Education and Research, CCCDI – UEFISCDI, project number PN-III-P2-2.1-PED-2019-2235, within PNCDI III.

## Methods

*Minimum inhibitory concentration (MIC)* was determined using a broth dilution method as described by CLSI M27 standard (2017).

*Cell membrane permeability* was evaluated using fluorescence microscopy and propidium iodine (PI) (Ma et all, 2020).

*Hyphal growth test* was performed using Leica DM100 LED microscope and scanning electron microscopy (Babii et all, 2016; Wang et all, 2017).

## Results

*Antimicrobial activity*. Synthetic flavonoid Br-Cl showed a very good antifungal activity, with MIC value of 15.62 µg/mL (Table 1).

*Membrane integrity analysis*. After 24 hours all cells exposed to the Br-Cl flavonoid become fluorescent due to the cell membrane damage (Figure 1 and Figure 2).

*Effect of Br-Cl flavonoid on the morphological transition from yeast to filamentous forms*. Microscopic observation showed that Br-Cl flavonoid (5 x CMI) inhibited yeast to hyphal transition (Figure 3).




**Figure 2** – Effect of Br-Cl flavonoid on membrane permeabilization by PI staining and fluorescence microscopy: A, B – control, C, D – cells incubated for 24 hours with Br-Cl flavonoid (5 x CMI).

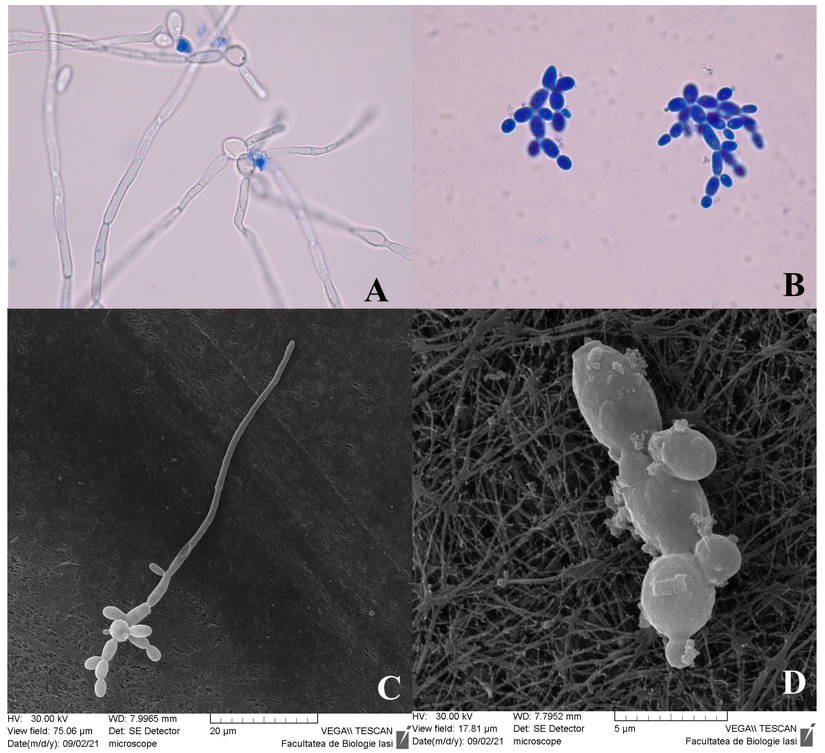



**Figure 3** – Effect of Br-Cl flavonoid on *C. albicans* yeast to hyphal transition. *C. albicans* was grown in RPMI medium in absence (A, B) or presence (C, D) of Br-Cl flavonoid. Inhibition of hyphal growth was visualized by light microscope, magnification 1000 x (A, B) and SEM (C, D).

# Analysis of the Lake Trout Heart and Blood Proteome using Evolutionary Proteomics

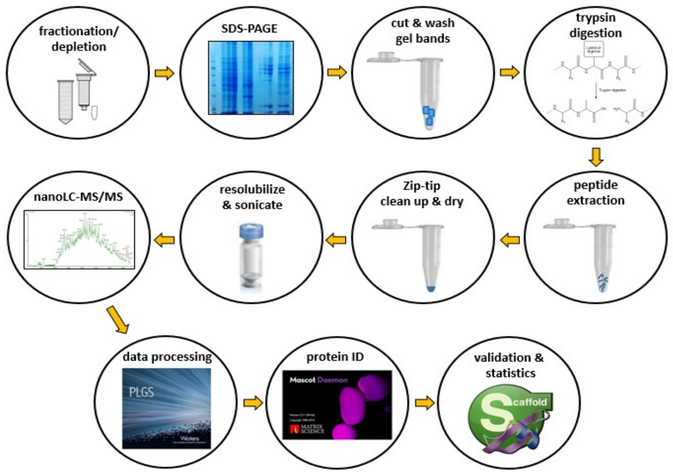
Shelby Alwine [1], Emmalyn J. Dupree [1], Bernard Crimmins [2], Thomas M. Holsen [3], Costel C. Darie [1]

[1]Biochemistry and Proteomics Group, Department of Chemistry & Biomolecular Science, Clarkson University, Potsdam, NY
[2]AEACS, New Kensington, PA [3]Department of Civil and Environmental Engineering, Clarkson University, Potsdam, NY

## Introduction

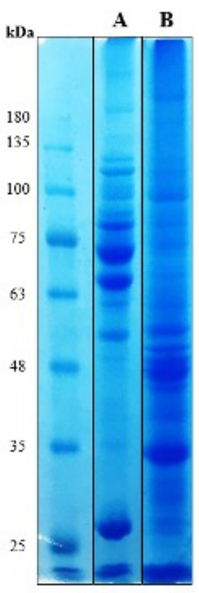
Persistent, bioaccumulative, and toxic chemicals (PBTs) are harmful chemicals that have been contaminating ecosystems for decades. In the Great Lakes scientists use lake trout to monitor the levels of PBTs present in the environment because lake trout are at the top of the food chain and therefore contain the high amounts of PBTs [1,2,3,4]. However, they would gain more knowledge about how PBTs are affecting the Lake Trout and the environment if there was a comprehensive database that contained all of the proteins in lake trout. Scientists could use the database to identify which biochemical pathways are functioning abnormally due to the PBTs, however no such database exists. The goal of this project is to begin identifying proteins in lake trout to start the process of building a protein database for this species and to identify evolutionary relationships for lake trout species.

## Methodology




## Results

**Figure 1 (A)**



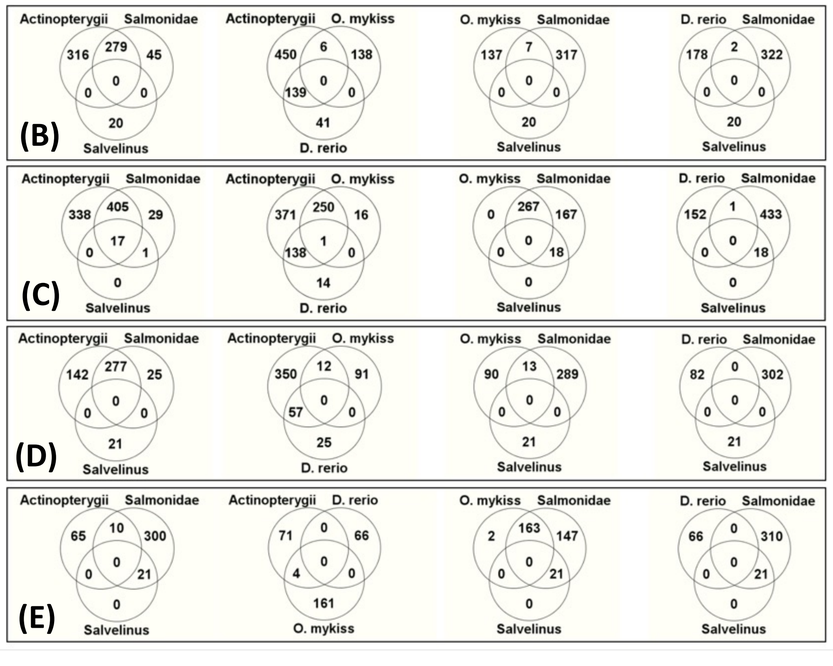
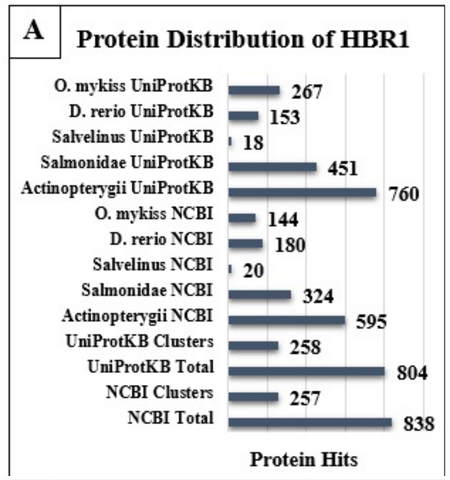




**Figure 1 (A):** SDS-PAGE containing heart (lane A) and blood (lane B) samples from a lake trout. **(B-E):** A representation of the similarity of the proteins found in the different databases for the first biological replicate. **(B)** Comparison of protein hits of HBR1 in the NCBI databases. **(C)** Comparison of protein hits of HBR1 in the UniProtKB databases. **(D)** Comparison of protein hits of BBR1 in the NCBI databases. **(E)** Comparison of BBR1 in the UniProtKB databases.



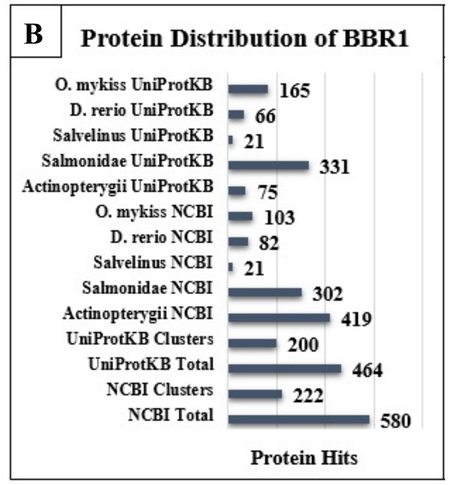




**Figure 2:** This shows the distribution of proteins that were identified in heart tissue and blood of lake trout for one biological replicate. Results were similar for a second biological replicate.
**(A):** Distribution of lake trout proteins identified by each database in the heart tissue sample.
**(B):** Distribution of lake trout proteins identified by each database in the blood sample.

## Conclusions

- We identified potential protein hits in the non-model species Salvlinus namaycush (lake trout), which could be used to build a comprehensive proteome database for this species.
  - In the heart tissue of biological replicate 1, 838 protein hits were identified in NCBI while 804 were identified in UniProtKB. In the blood there were 580 protein hits identified in NCBI and 464 identified in UniProtKB.
- Using protein databases for related species, peptides were identified that matched lake trout proteins and the proteins of other species. This allowed us to examine the evolutionary relationships between species.

## References

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# Impactul tratamentului cu plasmă non-termică la presiune atmosferică asupra expresiei genelor asociate controlului apoptozei

Stache Alexandru Bogdan[1,2], Dragoș Loredana Mihaiela[1,2], Topală Ionuț[3], Mihăilă Ilarion[3,4], Mihai Cosmin Teodor[5], Gorgan Dragoș Lucian[1]

[1]Facultatea de Biologie, Universitatea „Alexandru Ioan Cuza” din Iași, Iași, România. [2]Departamentul de Genetică Moleculară, Centrul de Cercetare Fundamentală și Dezvoltare Experimentală în Medicina Translațională - TRANSCEND, Institutul Regional de Oncologie, Iași, România. [3]Centrul de Cercetări Avansate în Fizica Plasmei din Iași - IPARC, Facultatea de Fizica, Universitatea „Alexandru Ioan Cuza” din Iași, Iași, România.
[4]Centrul Integrat de Studii în Știința Mediului pentru Regiunea de Dezvoltare Nord-Est - CERNESIM, Universitatea „Alexandru Ioan Cuza” din Iași, Iași, România.
[5]Centru Avansat de Cercetare-Dezvoltare in Medicina Experimentala - CEMEX, Universitatea de Medicina si Farmacie “Grigore T. Popa” din Iasi, Iasi, Romania.

**Introducere.** Osteosarcomul este un neoplasm osos malign ce apare ca o tumoră solidă producătoare de osteoid în metafiza oaselor lungi și este printre cele mai frecvente cauze de deces legate de cancer în rândul copiilor. Ca urmare a efectelor anti-tumorale selective observate, plasma non-termică la presiune atmosferică (CAP) a fost propusă ca potențial nou tratament în terapia cancerului. CAP este un gaz parțial ionizat compus din ioni, electroni, fotoni și elemente neutre, care sunt specii active capabile să inducă diferite fenomene fizice și reacții chimice. Scopul acestui studiu a fost de a evalua impactul expunerii indirecte la CAP a două modele celulare *in vitro* (osteoblaste și celule de osteosarcom) asupra expresiei unor gene asociate apoptozei.

## Materiale și metode

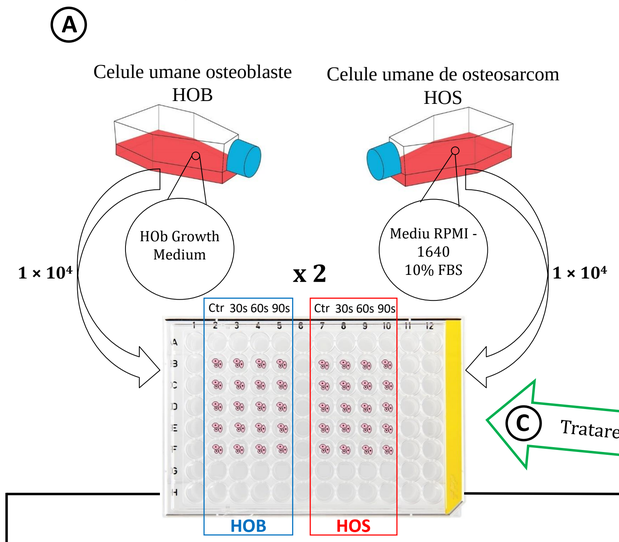


Figura 1. Reprezentarea schematică a distribuirii celulelor și a tratamentului în placă în vederea efectuării extracțiilor ARN și a testului de viabilitate

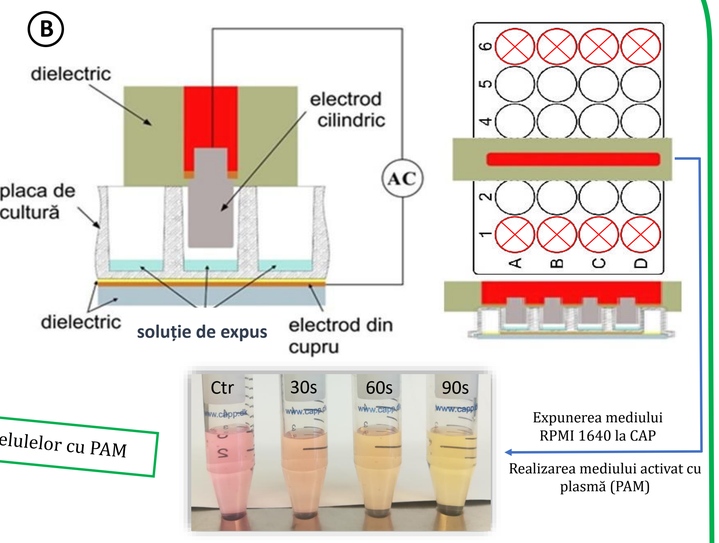


Figura 2. Reprezentare schematică a dispozitivului de descărcare a plasmei DBD, a poziției electrozilor în cazul unei plăci de culturi celulare cu 24 de godeuri și soluțiile PAM obținute

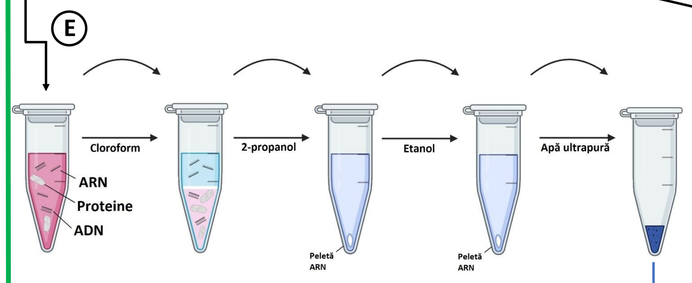


Figura 3. Izolarea ARN

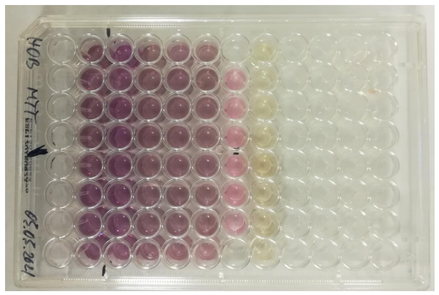

Figura 3. Evaluarea viabilității celulare prin testul colorimetric MTT

Figura 4. Analiza expresiei genice prin RT-qPCR

## Rezultate

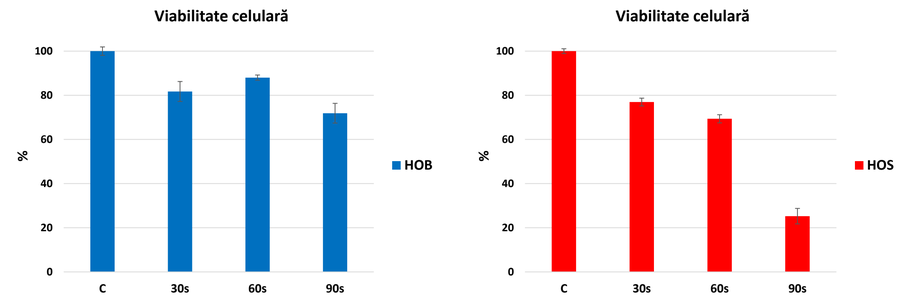


Figura 5. Evaluarea impactului tratamentului indirect cu CAP asupra viabilității celulelor HOB și HOS

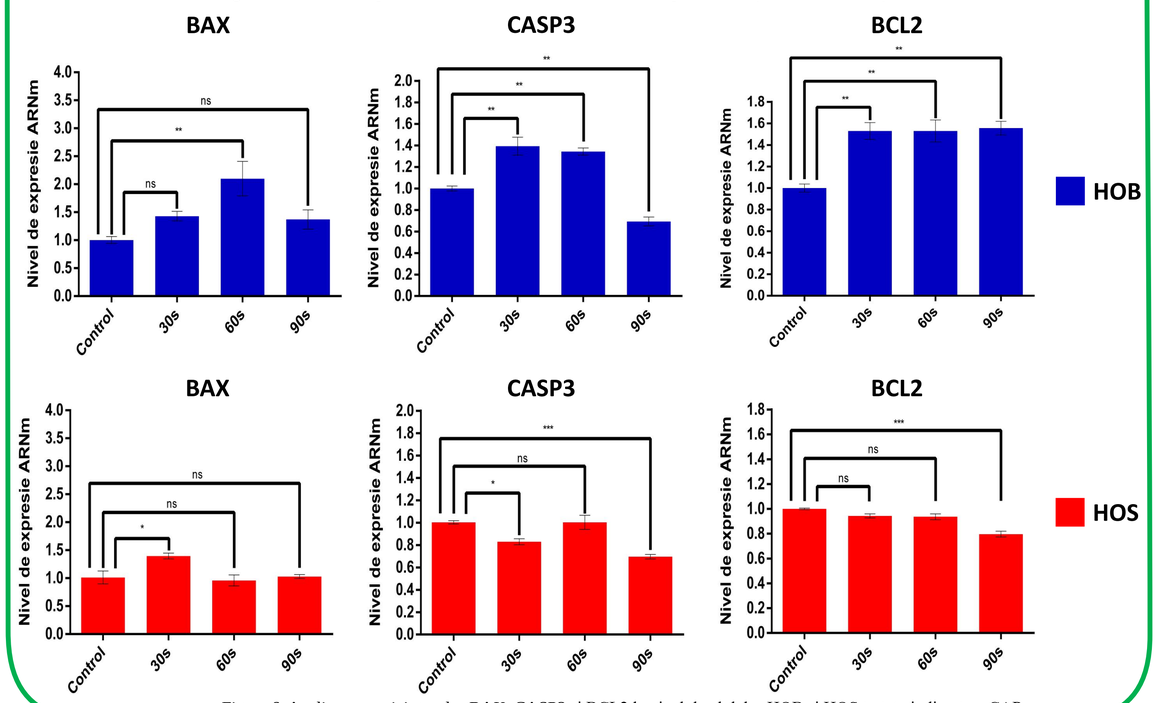


Figura 6. Analiza expresiei genelor BAX, CASP3 și BCL2 la nivelul celulelor HOB și HOS tratate indirect cu CAP

**Concluzii.** În cadrul acestui studiu, CAP prezintă un efect citotoxic selectiv. Tratamentul indirect activează calea apoptotică intrinsecă mediată de gena BAX și declanșează în celulele normale un mecanism molecular anti-apoptotic. Modificările nivelurilor de expresie genică nu sunt dependente de doza de tratament.

# MICROPROPAGATION FROM CALLUS CULTURES OF *CAPSICUM ANNUUM* L.

**Smaranda Vântu**[1]

[1] „Al. I. Cuza" University, Faculty of Biology, B-DUL CAROL I, 20 A, 700505 IASI, ROMANIA, s_vantu@yahoo.com

## Introduction

*Capsicum annum* L (Solanaceae) is an important source of chemical compounds such as vitamins, minerals, flavonoids, carotenoids, and capsaicinoids, especially capsaicin with high antioxidant, antimicrobial, anticancer, and analgesic capacity (Swetnisha, et al, 2017, Hernández-Pérez et al, 2020).

One of the biotechnological strategies of increasing secondary metabolites accumulation, with more advantages over extraction from fruits or artificial synthesis is plant tissue cultures (Ochoa et Ramirez, 2001).

Indirect micropropagation through callus cultures has become a biotechnological approach for the improvement of economically important plants.

The initiation of "in vitro" cultures aimed to evaluate the capacity of cell dedifferentiation and redifferentiation at *Capsicum annuum* l. species, depending on the origin of the explant and hormone stimuli in the culture medium (Valadez, et al., 2009, Hegde et al , 2017).

## Methods

The *Capsicum annum* L seeds were chemically sterilized, using a solution of 3% Na hypochlorite. Treatment duration was 15 minutes, followed by washing repeatedly with sterile distilled water.Surface sterilized seeds were aseptically inoculated on MS basal medium and incubated at 25±2 °C under 16h light and 8h dark photoperiod for raising the plantlets. The cultures of *Capsicum annum* L. were initiated on cotyledon and hypocotyl explants taken from in vitro grown 7 day old plantlets (Marta et Pawel, 2015). .

The proliferative capacity was tested on plantlets explants, cultivated on Murashige-Skoog basal medium, testing two auxins:: 2,4 dichlorphenoxyacetic acid (2,4 D) and β-indolylacetic acid (IAA) and a cytokinine: benzylaminopurine (BAP) (Table 1). The samples were kept in darkness.

The biomass accumulation was measured by regular weighing on analytical balance. For callus induction, it were tested some variants of MS medium, using β-indolylacetic acid (IAA) in combination with benzylaminopurine (BAP) in same concentration (variants 1) or 2,4 dichlorphenoxyacetic acid (2,4 D) used in combination with benzylaminopurine (BAP)., in same concentration (variants 3).For caulogenesis, it was tested a cytokinine: benzylaminopurine BAP) 1 mg/l with 0,1 mg/l β-indolylacetic acid IAA) (variants 2) or 0,1 mg/l 2,4 dichlorphenoxyacetic acid (2,4 D) (variants 4).

## Results

Table 1- Morphogenetical reactions of *Capsicum annuum* L., cultivated „in vitro"

| | Growth regulators mg/l | | | Morphogenetical reaction |
|---|---|---|---|---|
| | BAP | IAA | 2,4 D | |
| I | 1,00 | 1,00 | - | callus induction |
| II | 1,00 | 0,1 | - | caulogenesis |
| III | 1,00 | | 1,00 | callus induction |
| IV | 1,00 | - | 0,1 | caulogenesis |
| V | - | - | - | roots development |

BAP- benzylaminopurine: IAA- β-indolylacetic acid,

2, 4 D – 2,4 diclorphenoxyacetic acid

Fig .1, 2- Stages of cell dedifferentiation

Fig. 3,4 Primary callus culture from hypokotyl explant

Fig. 5 -Early stage of caulogenesis

Fig. 6- Shoots development

Fig. 7- Multiple shoots development

Fig. 8- In vitro regenerated plant

Fig. 9 – Acclimatisation of regenerated plant

## Conclusions

1. The callus induction was achieved from hypocotyl explant on MS medium,supplemented with 1.0 mg/l IAA and 1.0 mg/l BAP.
2. The caulogenesis induction was performed on MS medium, supplemented with 1 mg/l BAP – benzylaminopurine in combination with 0,1 mg/l IAA – β-indolylacetic acid or 0,1 mg/l 2.4 D- 2,4 dichlorphenoxyacetic acid.
3. The regeneration of whole plants was obtained in two steps: the shoots were excised and transferred to MS fresh medium and then roots development was achieved on the same medium without growth regulators.