From Nicotine-Containing Waste to Value-Added Chemicals: The Case of *Paenarthrobacter nicotinovoransrans*

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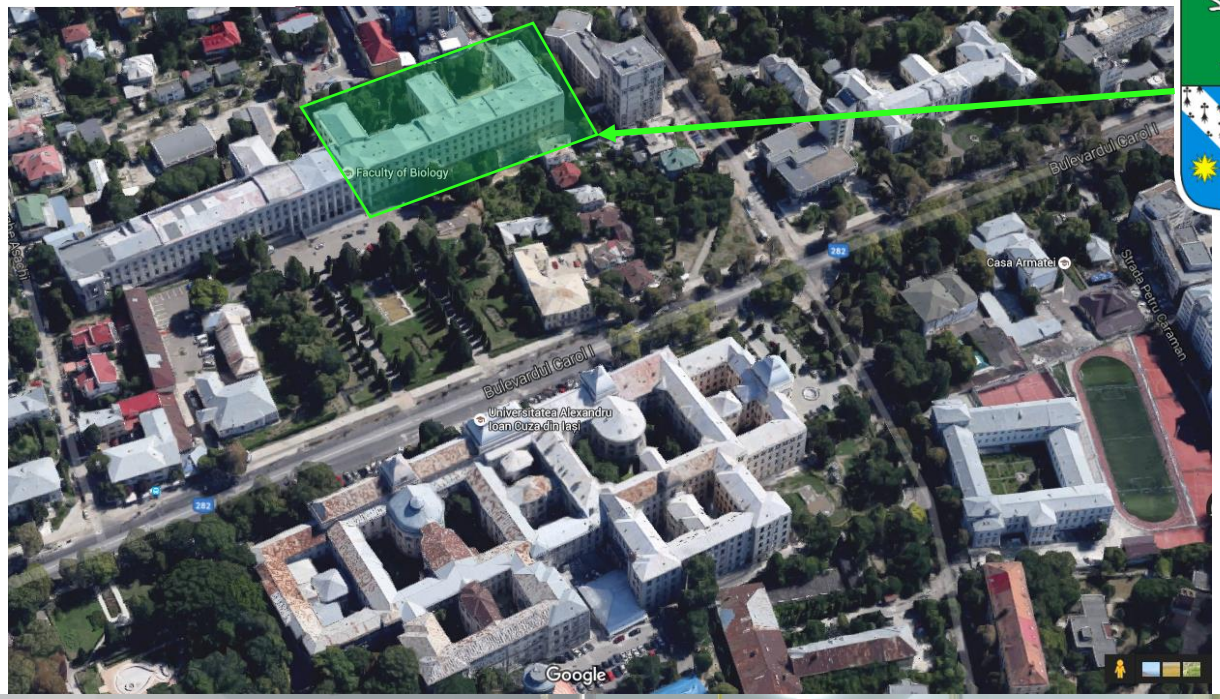
A bit about us and our work in Iași

Biology Department, Alexandru Ioan Cuza University of Iași, Romania

Department of Biology



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BioActive research group

- Isolation
- Identification
- Characterization
- Biological effects (neurological effects, citotoxycity, oxidative stress, antimicrobial activity

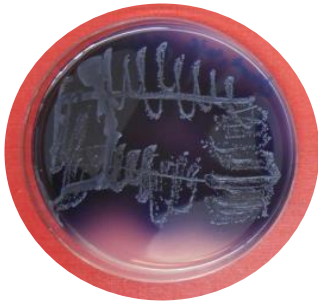
of biological active molecules with potential applications in biotechnology.

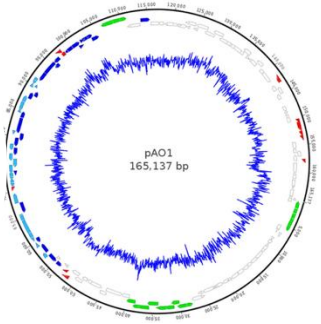
A bit about us and our work in Iași

Biology Department, Alexandru Ioan Cuza University of Iași, Romania

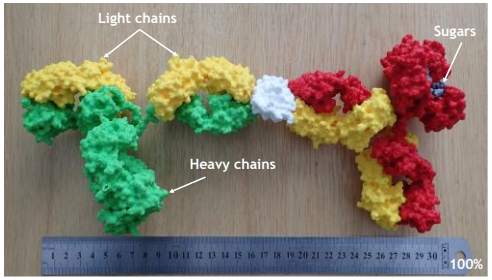
Main research subjects:

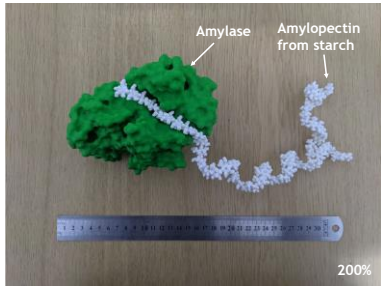
- molecular biology of pAO1 megaplasmid related to nicotine catabolism, stress induced by nicotine degradation and biotechnological applications..



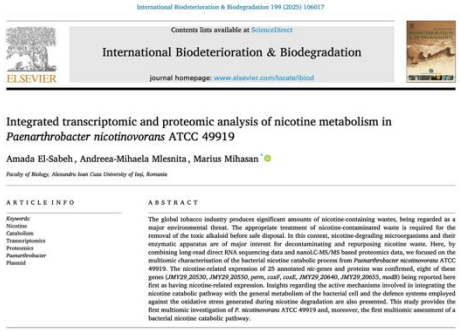


- using 3D printing for creating teaching materials to support molecular bioscience education.





Latest paper:



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journal homepage: www.elsevier.com/locate/ibiod

Integrated transcriptomic and proteomic analysis of nicotine metabolism in *Paenarthrobacter nicotinovorans* ATCC 49919

Amada El-Sabeh, Andreea-Mihaela Mlesnita, Marius Mihasan

ARTICLE INFO

ABSTRACT

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Thu 6.11, 9:55 – 10:15
Andreea Mihaela Mlesnita

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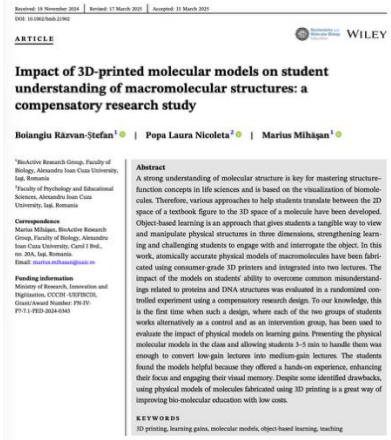
Latest paper:



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DOI: 10.1002/bmb.21902

ARTICLE

WILEY

Impact of 3D-printed molecular models on student understanding of macromolecular structures: a compensatory research study

Boiangiu Răzvan-Ștefan | Popa Laura Nicoleta | Marius Mihășan

Abstract

KEYWORDS

10.1002/bmb.21902

Developed tools:

Jmol/JSmol script for automatic generation of 3D printable molecular models from structural data.

ICMB2025

Fri 07.11, 11:10

Paenarthrobacter nicotinovorans

Biology Department, Alexandru Ioan Cuza University of Iași, Romania

J Biol Chem. 1959 Jan;234(1):151-5.

The bacterial oxidation of nicotine. I. Nicotine oxidation by cell-free preparations

J Biol Chem. 1972 Dec 10;247(23):7628-33.

The bacterial oxidation of nicotine. 8. Synthesis of 2,3,6-trihydroxypyridine and accumulation and partial characterization of the product of 2,6-dihydroxypyridine oxidation

J Bacteriol. 2003 Mar;185(6):1976-86. doi: 10.1128/JB.185.6.1976-1986.2003.

Sequence of the 165-kilobase catabolic plasmid pAO1 from Arthrobacter nicotinovorans and identification of a pAO1-dependent nicotine uptake system

J Mol Evol. 2013 Aug;77(1-2):22-30. doi: 10.1007/s00239-013-9576-x.

pAO1 of Arthrobacter nicotinovorans and the spread of catabolic traits by horizontal gene transfer in gram-positive soil bacteria

Marius Mihasan [1], Roderich Brandsch

Affiliations + expand

PMID: 23884627 DOI: 10.1007/s00239-013-9576-x

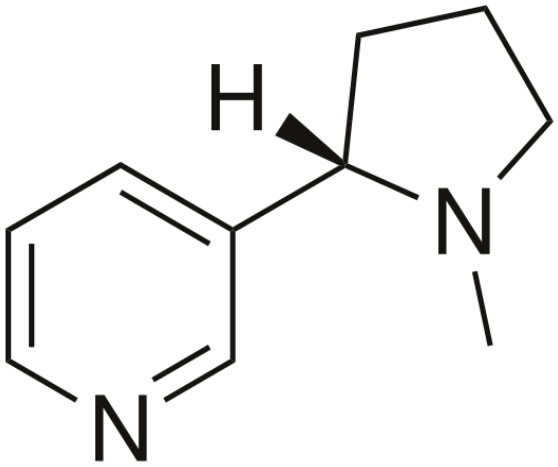
Nicotine - a lead compound for developing drugs

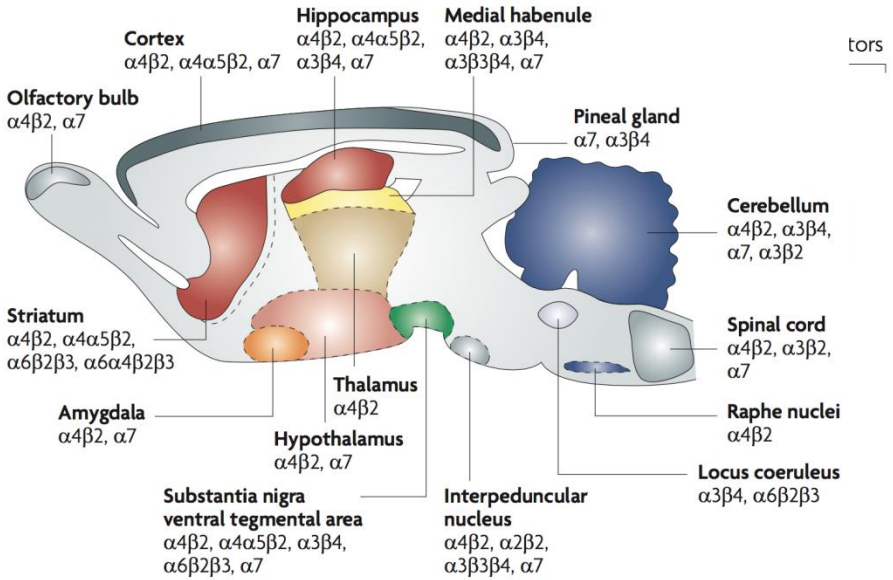
Biology Department, Alexandru Ioan Cuza University of Iași, Romania

Nicotiana tabacum

Nicotine

- Produced by the **tobacco plant** to prevent consumption by herbivores
- **Responsible for smoking addiction** - by releasing dopamine
- **Potentially lethal to humans** (LD_{50} 0.5 – 1.0 mg/kg)
- **Improves: cognition, alertness, memory**





nAChRs - Nicotine acetylcholine receptors

ligand-gated ion channel superfamily of neurotransmitter receptors

Nicotine is a lead compound used for developing drugs for cognitive dysfunctions

Levin ED and Rezvani AH. Nicotinic treatment for cognitive dysfunction. Curr DrugTargets CNS Neurol Disord. 2002;1(4):423-31.
Taly et. all. Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system Nat. Rev. Drug Discov.2009, 8, 733-750.

6-hydroxy-L-nicotine – a neuroprotective agent

Biology Department, Alexandru Ioan Cuza University of Iași, Romania

Nicotine binds to **nAChRs subtypes α4β2 -**

Murray and Abeles, *Aging & Mental Health.* 2002, 6, 129–138.

Could

Paenarthro

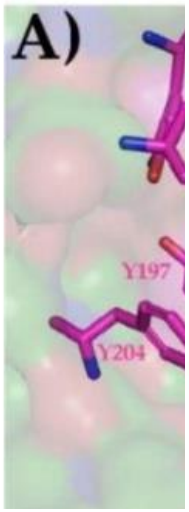
***In silico* - Targ**

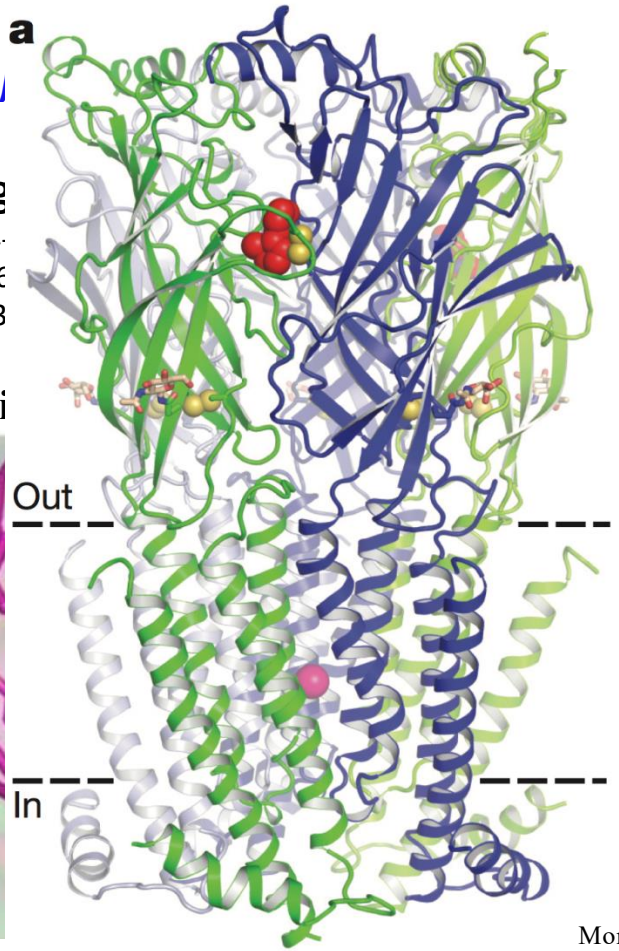
– rigid receptor - α4

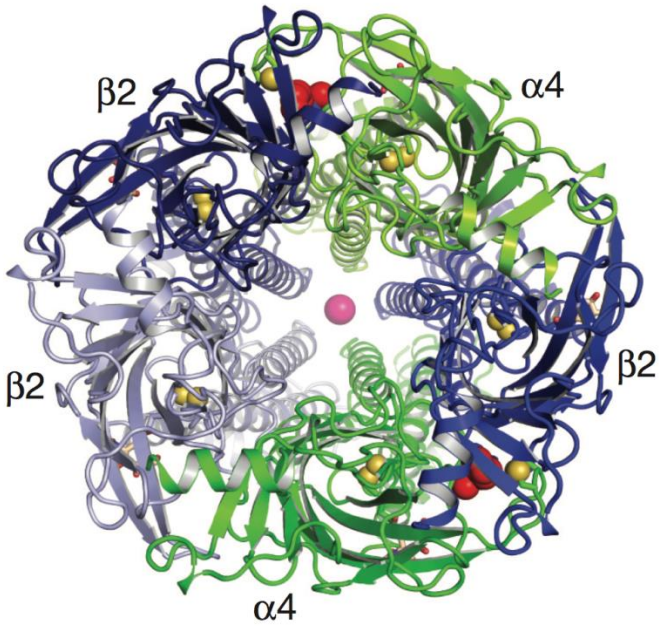
- targeted region - 6

- flexibile ligands – 3

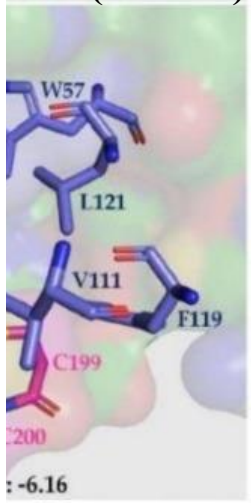
(S)-Nicoti







otine(docked)



Morales-Perez C et al., *Nature*, 2016, 538 (7625): 411–415

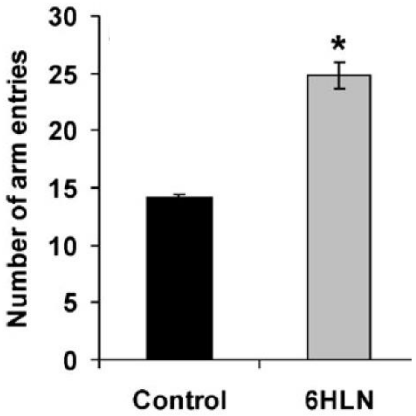
10.3390/antiox10020212

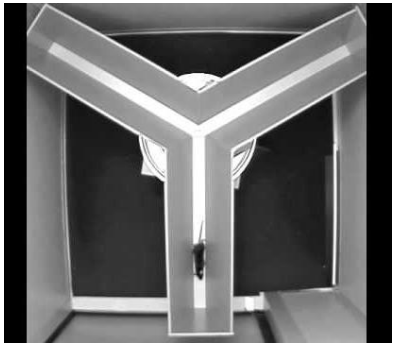
6-hydroxy-L-nicotine – a neuroprotective agent

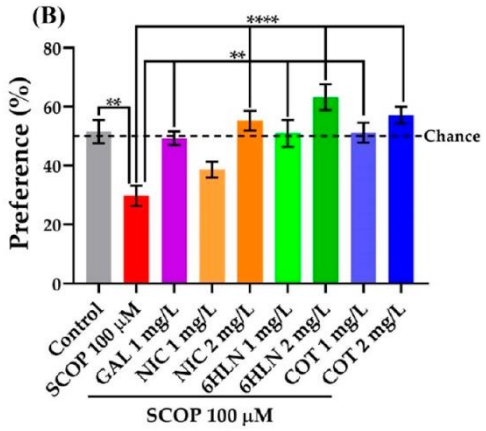
Biology Department, Alexandru Ioan Cuza University of Iași, Romania

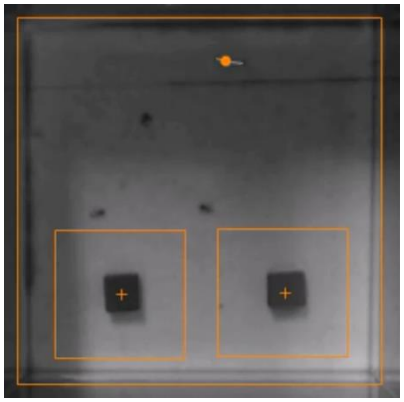
In-vivo tests

- male Wistar **rats** (3-4 months old)
- **zebrafish** (*Danio rerio*), both sexes (50:50)



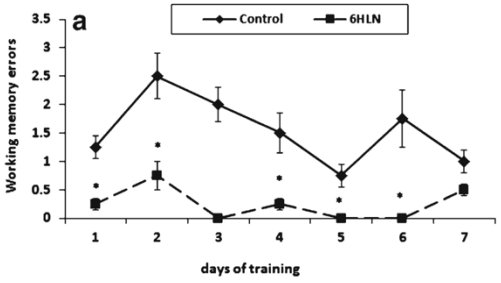


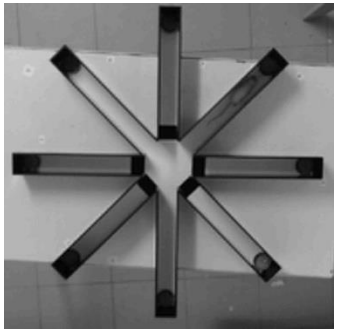




Y-maze task

Values are mean ± S.E.M. (n=10 animals per group), *p<0.0001 vs. control group





Object Discrimination Test

- Values are mean ± S.E.M. (n=10 animals per group), $F(8,81) = 8.078$, $p < 0.0001$. For Tukey posthoc analyses—**** $p < 0.0001$, and ** $p < 0.001$

Prof. Lucian H., PhD

Radu I.

Razvan B.

Radial arm-maze task

Values are mean ± S.E.M. (n=10 animals per group), *p<0.0001 vs. control group

10.1016/j.biopha.2016.12.008

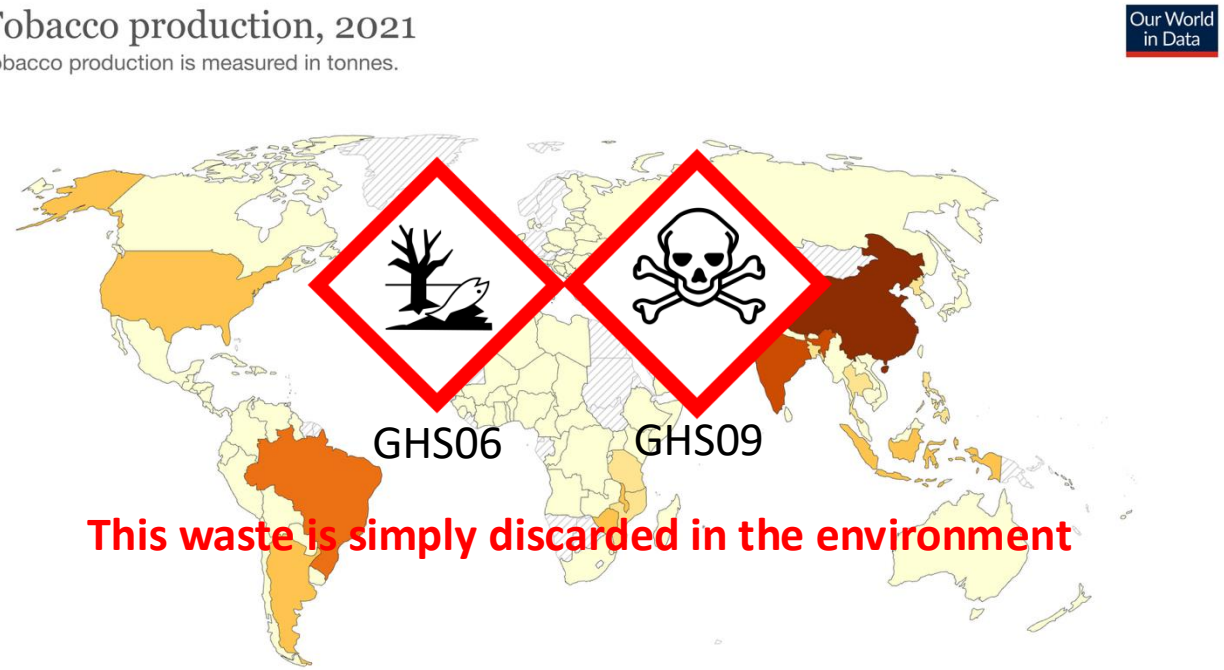
10.3390/antiox9080768

Nicotine-containing waste need to be decontaminated

Biology Department, Alexandru Ioan Cuza University of Iași, Romania

Tobacco industry produces 300 000 tons of non-recyclable nicotine-containing waste per year, 18 g nicotine/per kg

- According to EPA the waste is **Toxic Release Inventory (TRI)** chemicals
- According to the European Union Regulations (EUR) - **"toxic and hazardous"**(when nicotine content is over 0.05% w/w)



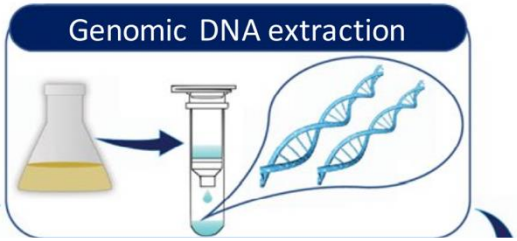
This waste is simply discarded in the environment

Why not using *Paenarthrobacter nicotinovorans* to decontaminate this waste and convert it to useful chemicals?

Sequencing the *Paenarthrobacter nicotinovorans* genome

Biology Department, Alexandru Ioan Cuza University of Iași, Romania

Short reads sequencing

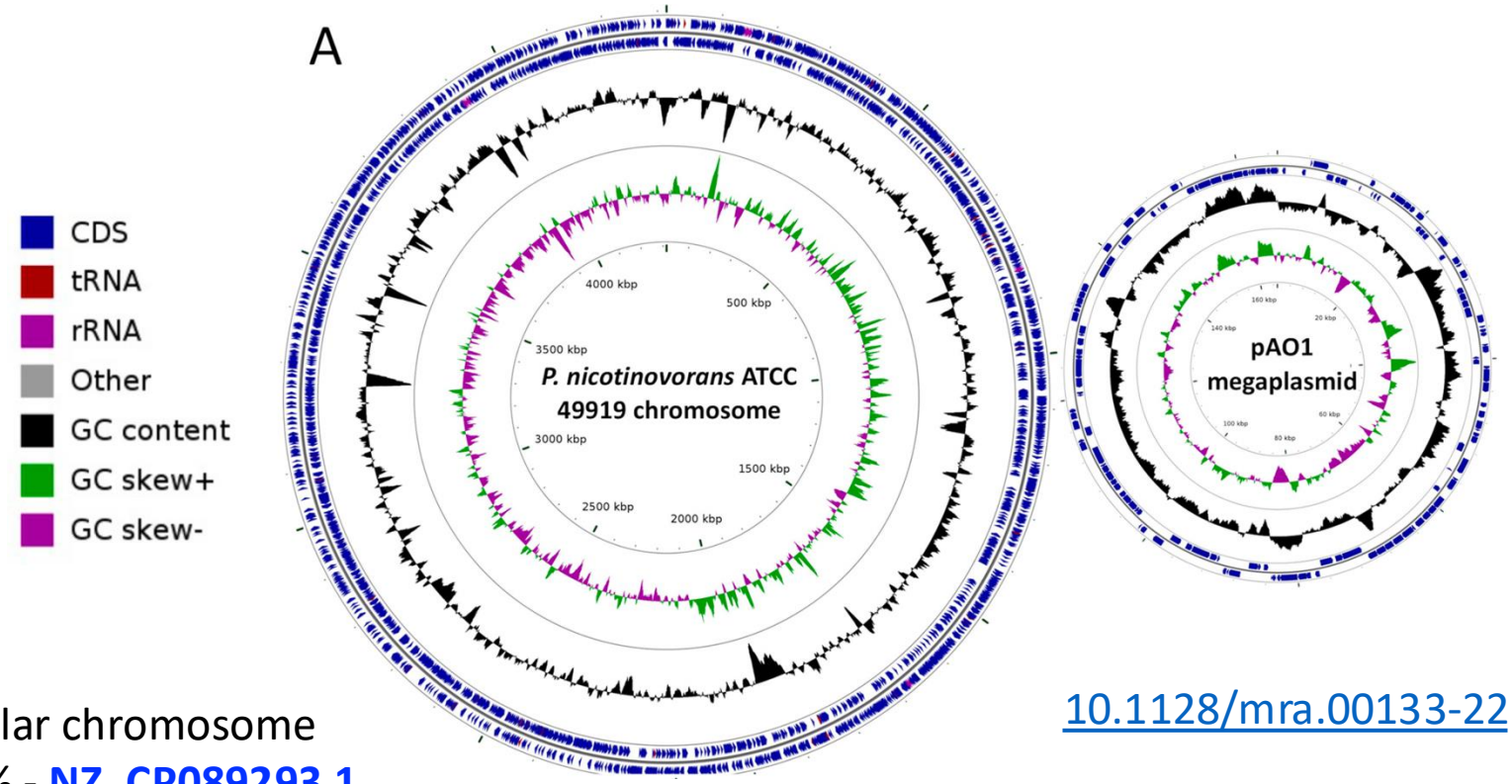


Long reads sequencing

Modified from - Ravintheran, S.K., Sivaprakasam, S., Loke, S. *et al.* Complete genome sequence of *Sphingomonas paucimobilis* AIMST S2, a xenobiotic-degrading bacterium. *Sci Data* **6,** 280 (2019)

Sequencing the *Paenarthrobacter nicotinovorans* genome

Biology Department, Alexandru Ioan Cuza University of Iași, Romania



4 316 184-bp circular chromosome
GC content - 63.2% - **NZ_CP089293.1**
165 141-bp circular megaplasmid
GC content - 59.7% - **NZ_CP089294.1**

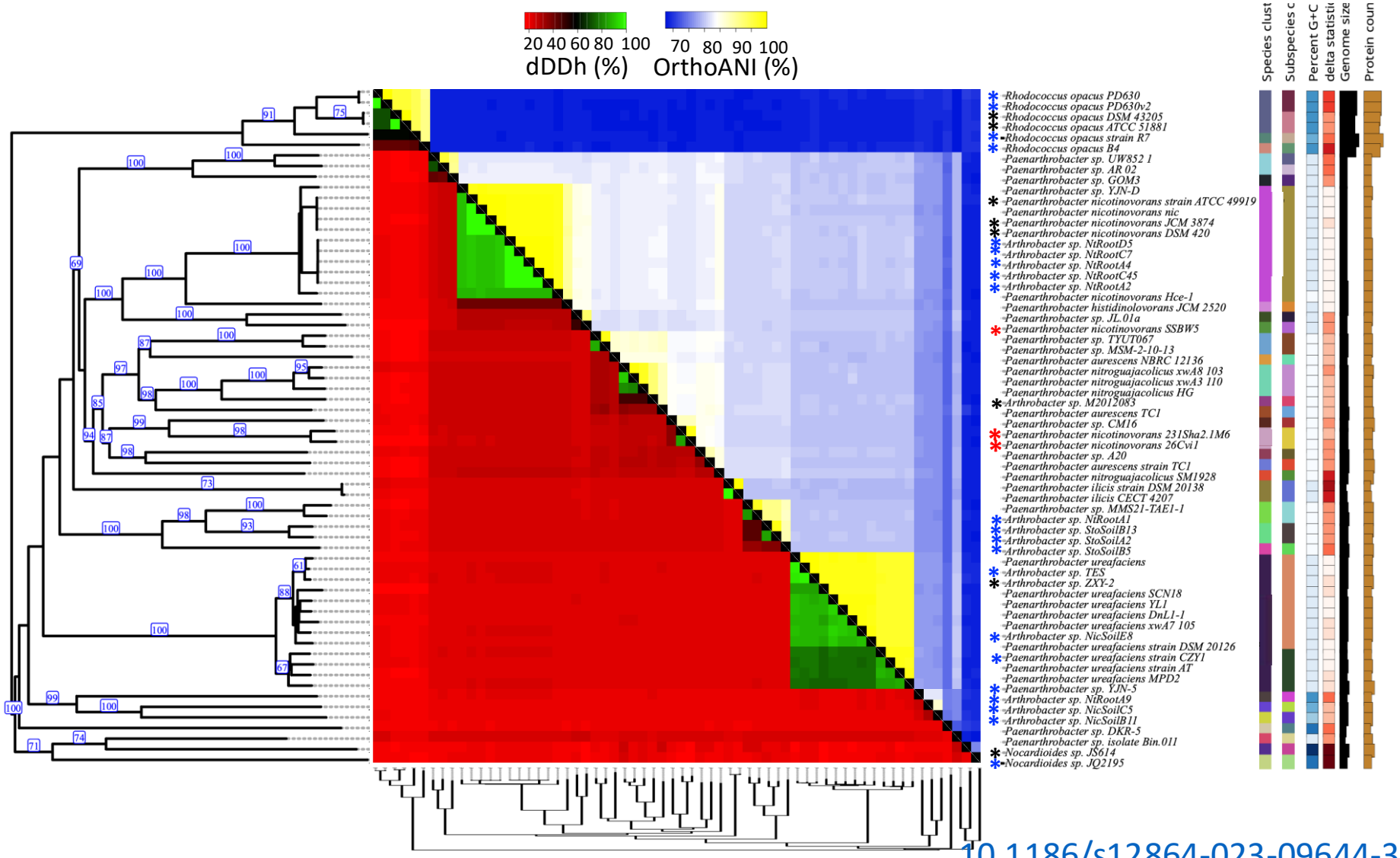
CheckM analysis
Completeness: 85.21%
Contamination: 10.14%

10.1128/mra.00133-22

Amada ES.

Phylogenomic analysis of 64 *Paenarthrobacter, Arthrobacter, Nocardioides* and *Rhodococcus* strains

Biology Department, Alexandru Ioan Cuza University of Iași, Romania



10.1186/s12864-023-09644-3

nic-genes cluster as a putative catabolic transposon

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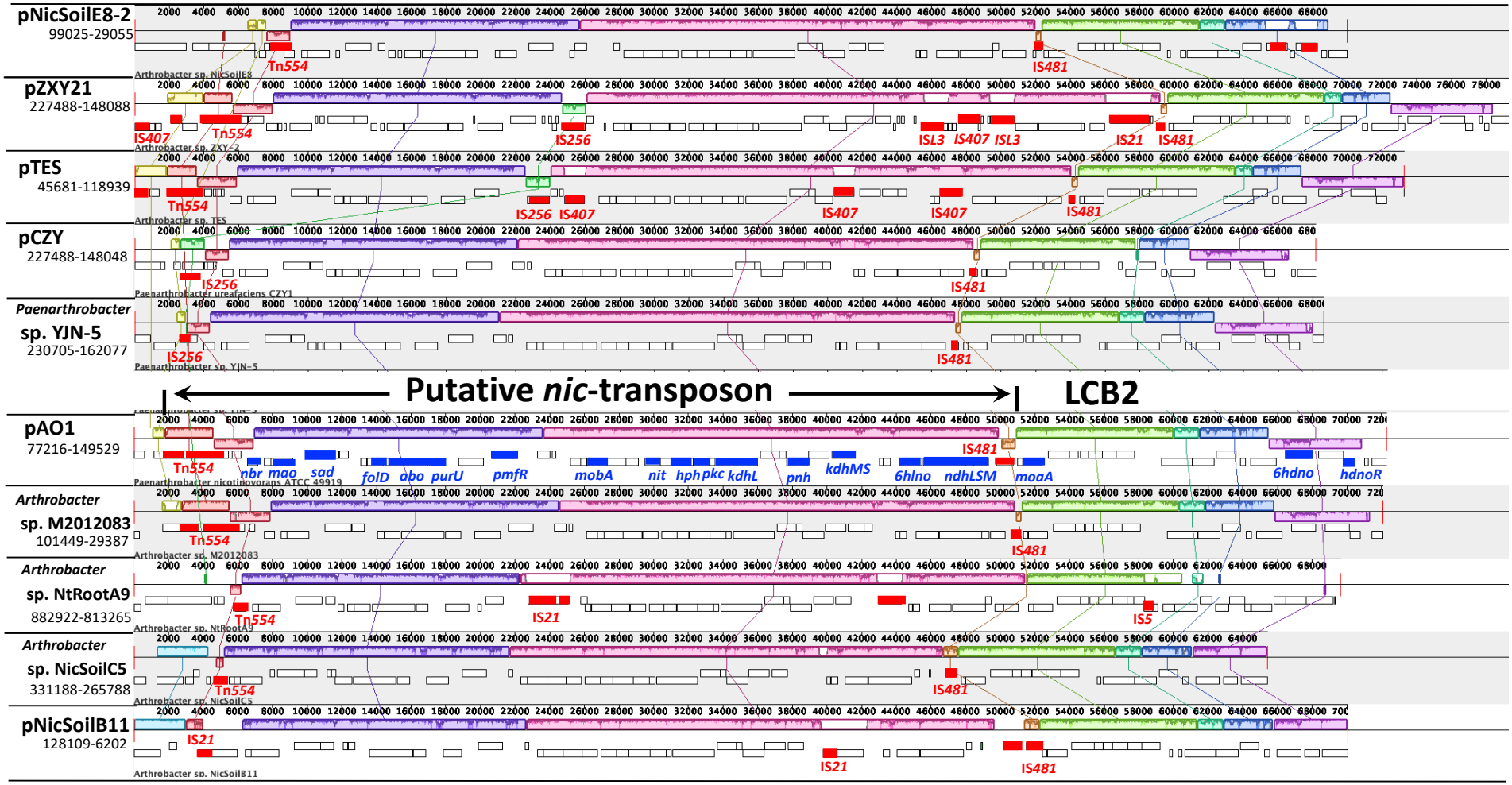

10.1186/s12864-023-09644-3

But when and which genes are expressed when nicotine is present?

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Total RNA isolation

↓

PolyA tailing

using a *E. coli* Poly(A) Polymerase I (E-PAP)

↓

Direct RNA sequencing on the Flongle/MinION device

using the SQK-RNA002 from Nanopore

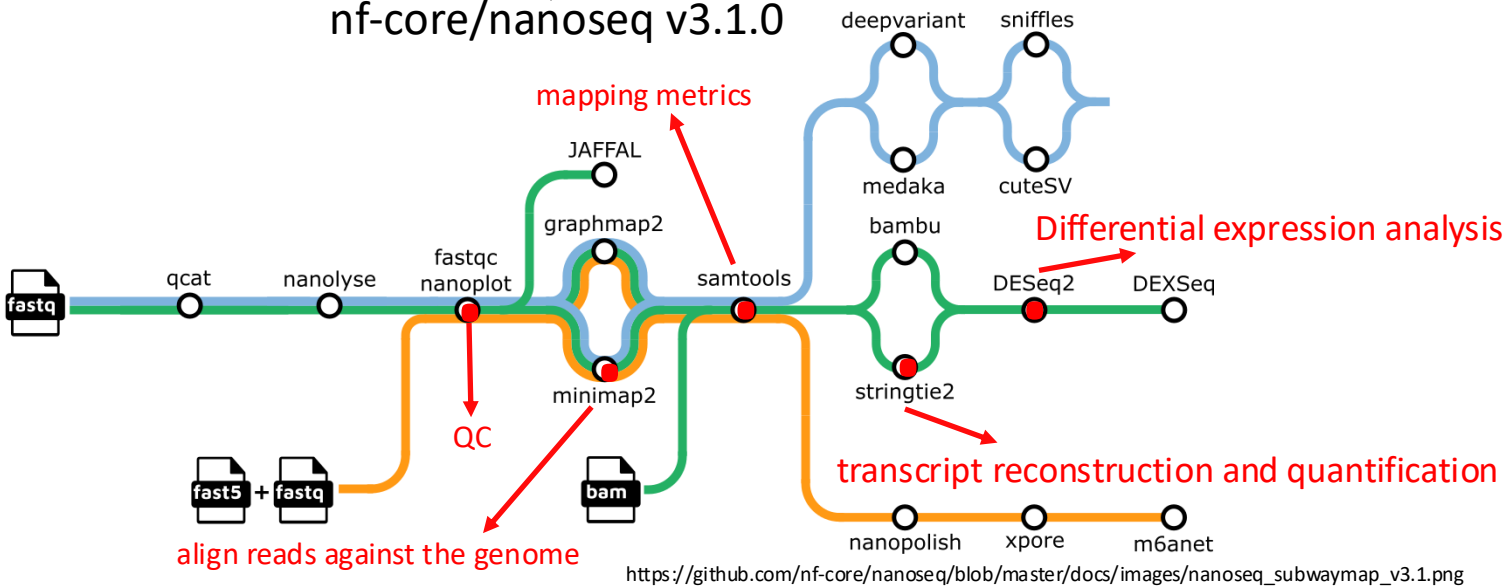
↓

Basecalling:

Guppy v.6.3.2, rna_r9.4.1_70bps_hac.cfg

↓

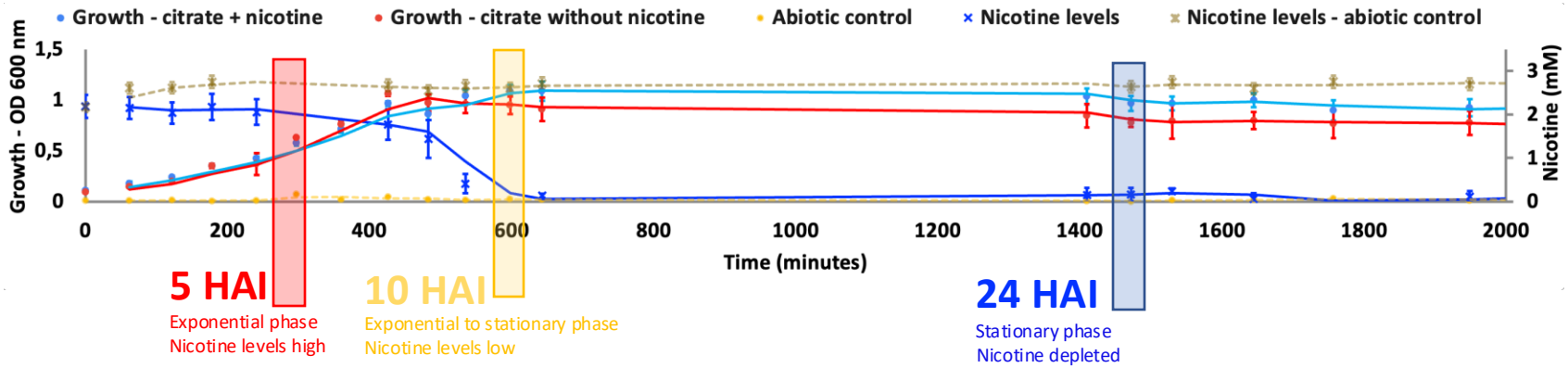
nf-core/nanoseq v3.1.0



https://github.com/nf-core/nanoseq/blob/master/docs/images/nanoseq_subwaymap_v3.1.png

Nicotine-related transcriptome of *Paenarthrobacter nicotinovorans*

Biology Department, Alexandru Ioan Cuza University of Iași, Romania



Differentially expressed genes (citrate vs citrate + nicotine)

	pAO1 nic-genes cluster			Chromosome encoded		
	5 HAI	10 HAI	24 HAI	5 HAI	10 HAI	24 HAI
pADJ < 0.1; |log2FoldChange|>1	*nbr, mao, sad, abo,* ***coxF****,* ***coxD****, nit,* ***coxG****, hph,* ***pkc****,* ***hyp****, kdhL, pnh, kdhSM, 6-hlno, ndhLSM,* ***moaA****,* ***moaE****,* ***modA****,* ***modB****,* ***modC***	*nbr, mao, sad, abo,* ***coxD****, nit,* ***coxG****, hph,* ***pkc****,* ***hyp****, kdhL, pnh, kdhSM, 6-hlno, ndhLSM,* ***moaE****,* ***modA****,* ***modB****,* ***modC***	nd	**site-specific integrase; argininosuccinate synthase; helix-turn-helix transcriptional regulator; Fis family transcriptional regulator;** ***argB*** **(acetylglutamate kinase); acetylornithine transaminase;** ***argF*** **(ornithine carbamoyltransferase); arginine repressor; ATP-dependent chaperone ClpB; RNAs and ribosomal proteins**	**site-specific integrase; argininosuccinate synthase; helix-turn-helix transcriptional regulator; Fis family transcriptional regulator; universal stress protein; tripartite tricarboxylate transporter permease; tripartite tricarboxylate transporter TctB family protein; tripartite tricarboxylate transporter substrate binding protein;**	nd

Bold – genes not previously shown to have nicotine related expression;
Red – genes differentially expressed between 5 HAI and 10 HAI.

P. nicotinovorans – nicotine induced proteome

Biology Department, Alexandru Ioan Cuza University of Iași, Romania

Growth, harvest and cell lysis

0.05% nicotine No nicotine

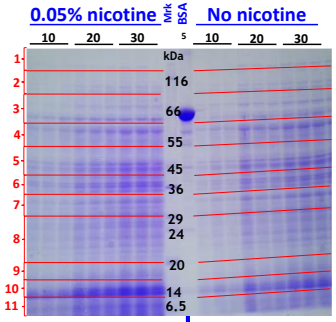
Lysozyme
SDS
DTT

12,000 g
30 min

Soluble proteins

Protein preparation

Protein fractionation
SDS-PAGE maxi
9-16% gradient gels

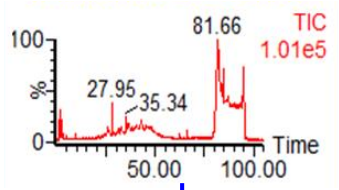


In-gel digestion

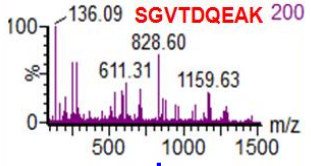
Peptide mixture

Identification and data analysis

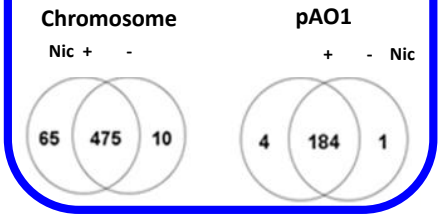
Nano-liquid chromatography tandem mass spectrometry



Protein identification by mass spectrometry



Data analysis: MASCOT; SCAFFOLD



Darie Costel, PhD
Clarkson University
Potsdam, NY, USA

Devika C.

Emma D.

Roshi A.

FULBRIGHT ROMANIA

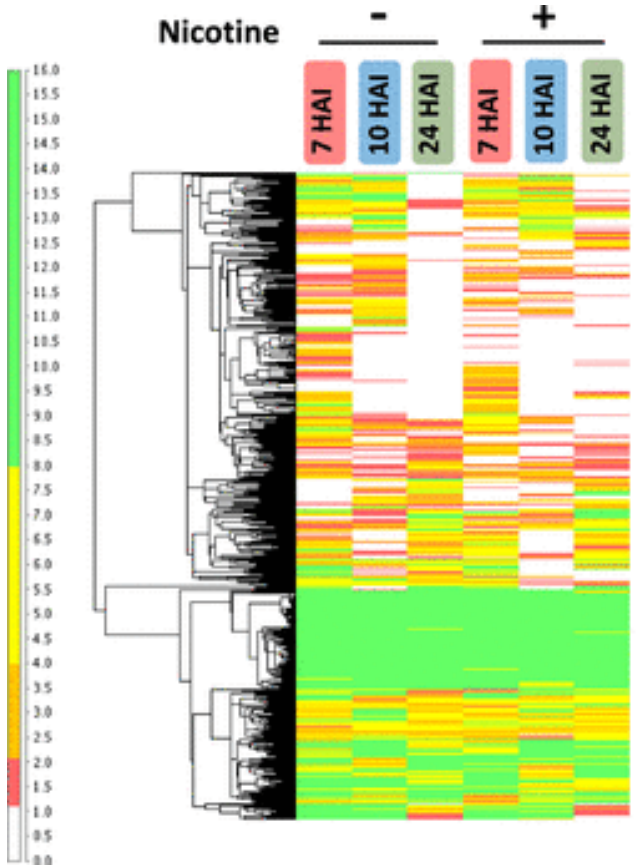
P. nicotinovorans – nicotine induced proteome

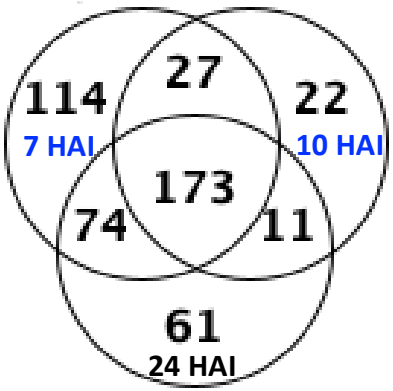
Biology Department, Alexandru Ioan Cuza University of Iași, Romania

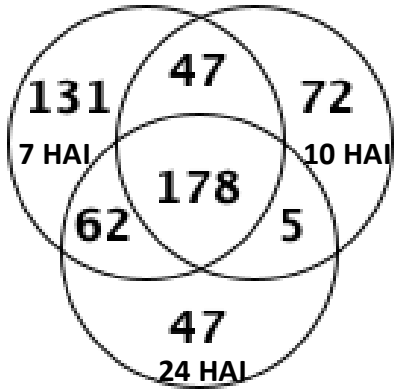
44136 MS/MS spectras assigned
2 peptides minimum

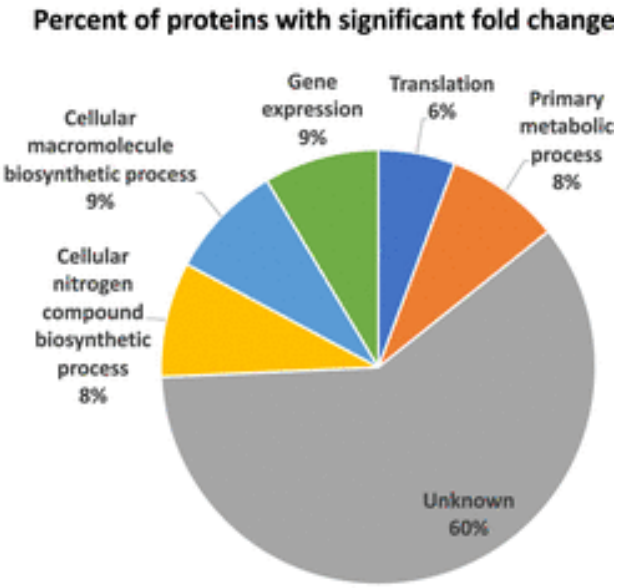
607 proteins at 1% FDR

ProteomeXchange: PXD012577





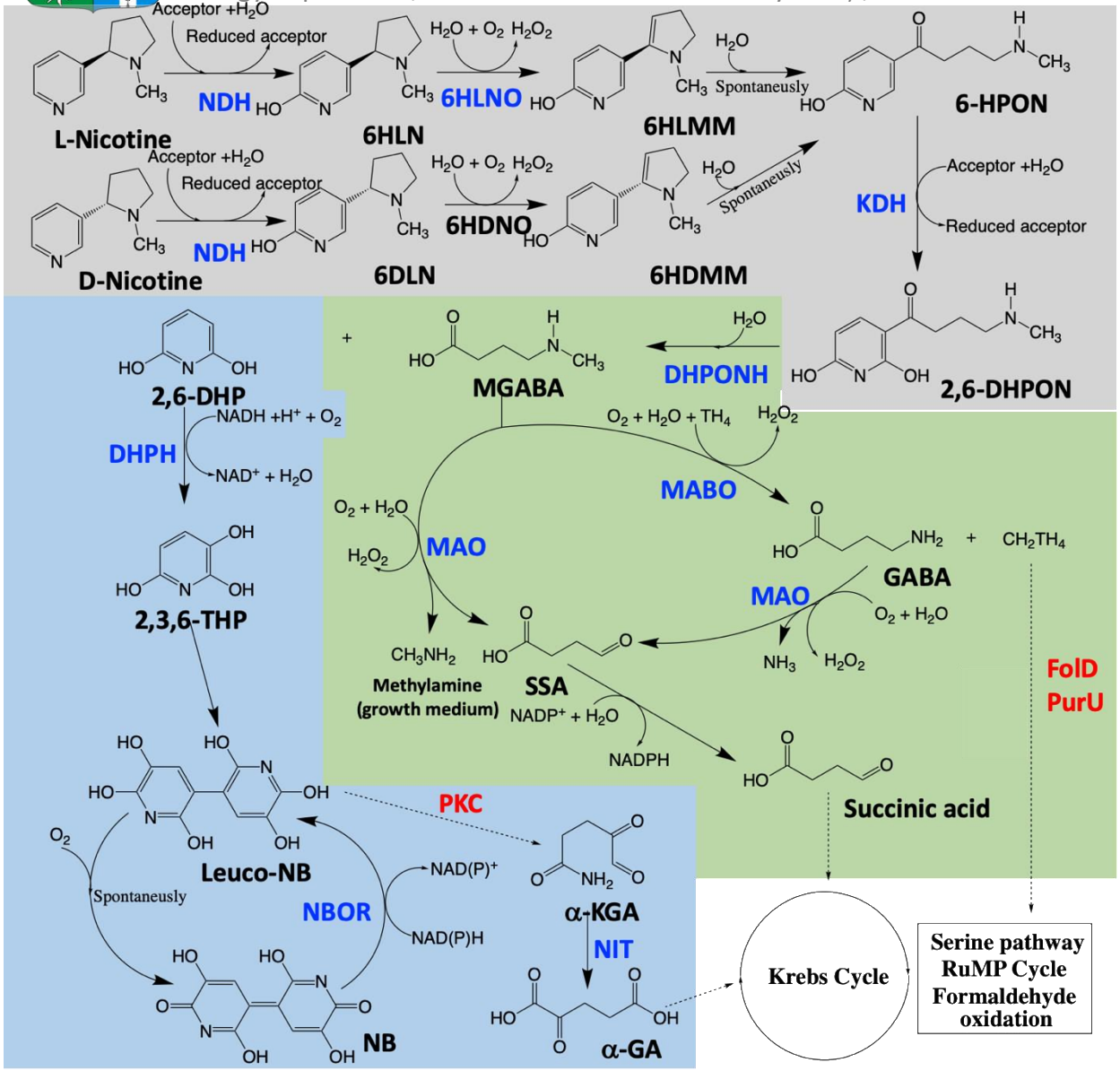




Marius Mihășan et. al. *ACS Omega* **2021** *6* (22), 14242-14251 DOI: 10.1021/acsomega.1c01020

Proteomic and transcriptomic view over the nicotine degradation pathway

Biology Department, Alexandru Ioan Cuza University of Iași, Romania



Detected by MS/MS and transcriptomics, known
to be involved in nicotine metabolism

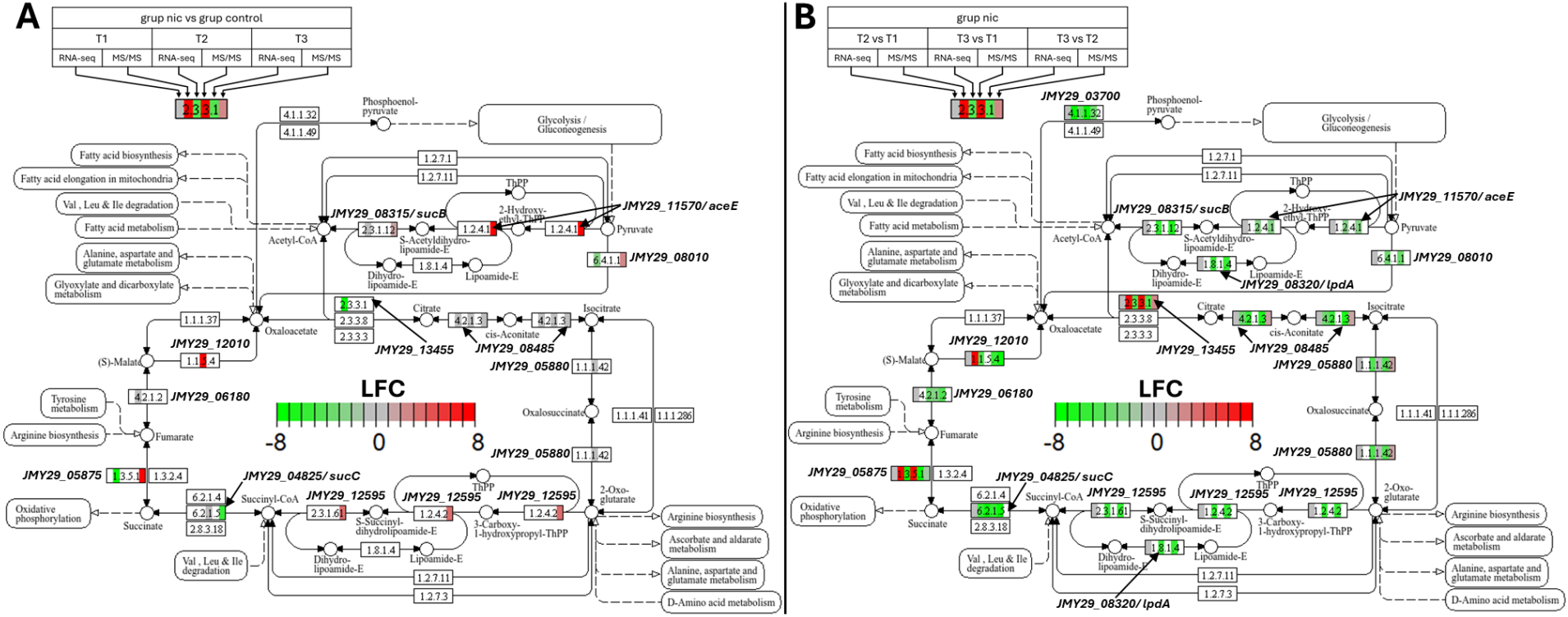
Detected by MS/MS and transcriptomics, putatively involved
in nicotine metabolism

Detected by MS/MS and transcriptomics,
CoxF
CoxD
CoxG
MoaA
MoaE
ModA
ModB
ModC
Molybdenum intake / cofactor / multimeric enzyme assembly

Integration of transcriptomics and proteomics data

Biology Department, Alexandru Ioan Cuza University of Iași, Romania

PATHVIEW



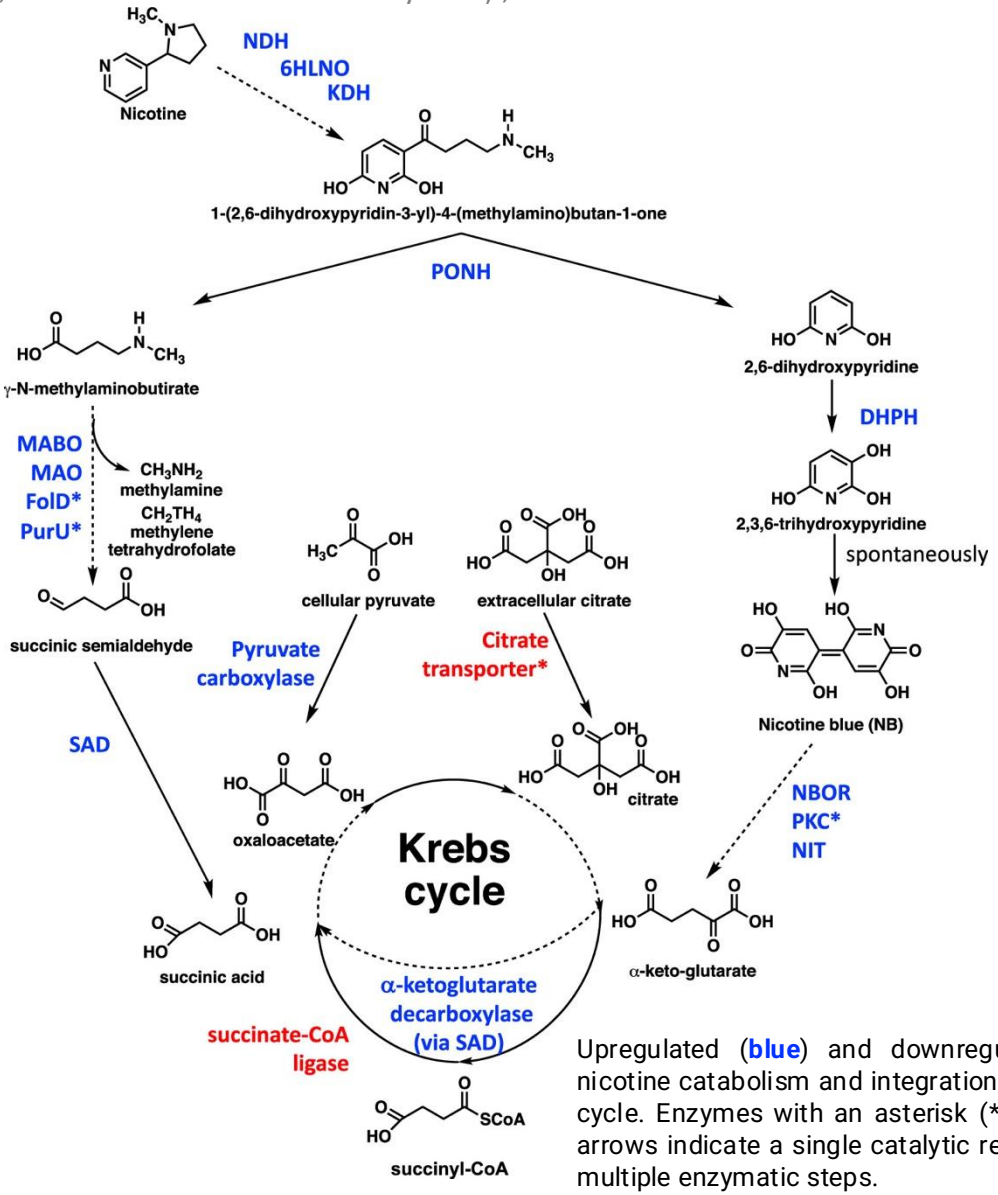
Differentially expressed genes (DEGs) and proteins (DEPs) involved in TCA:

A. nicotine vs control;
B. time course analysis in the nicotine-induced cells; LFC: log2FoldChange.;

10.1016/j.ibiod.2025.106017

Nicotine metabolism integration

Biology Department, Alexandru Ioan Cuza University of Iași, Romania



Upregulated (**blue**) and downregulated (**red**) proteins involved in nicotine catabolism and integration of the end products into the Krebs cycle. Enzymes with an asterisk (*) have putative functions. The full arrows indicate a single catalytic reaction; the dashed arrows indicate multiple enzymatic steps.

A *P. nicotinovorans* based biotechnology to produce 6HLNic

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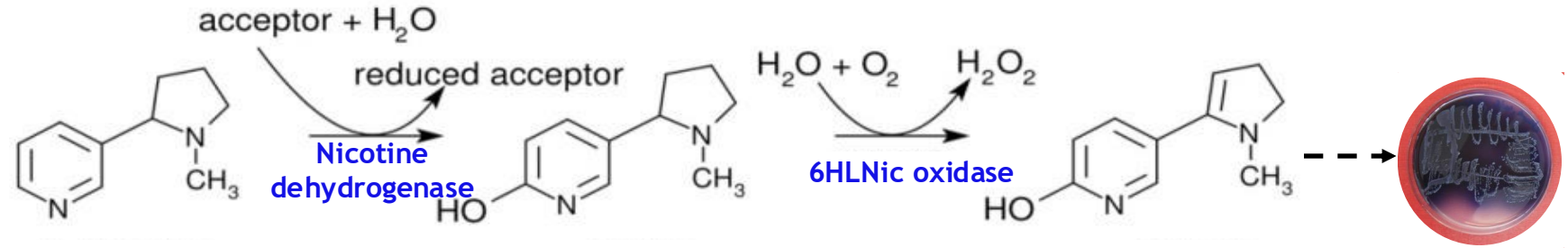
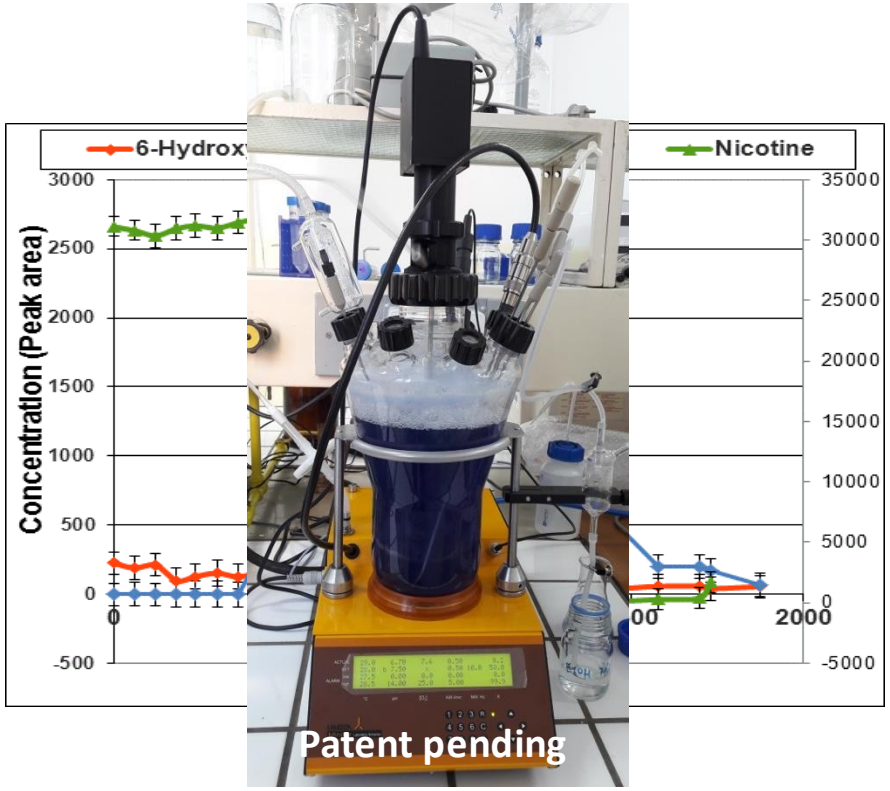


L-Nicotine

6-hydroxy L-Nicotine

6-OH, CH_3-Myosmine

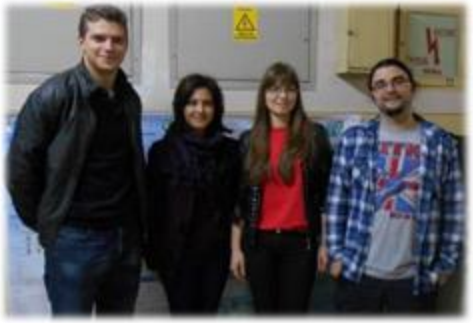


Diana M., B.Sc.

Ana I., B.Sc.

Andreea P, B.Sc.

Carmen C, B.Sc.



Razavn B., B.Sc

Doina G. B.Sc

Andreea A., M.Sc

Madalina M., B.Sc.

Loredana C., B.Sc.

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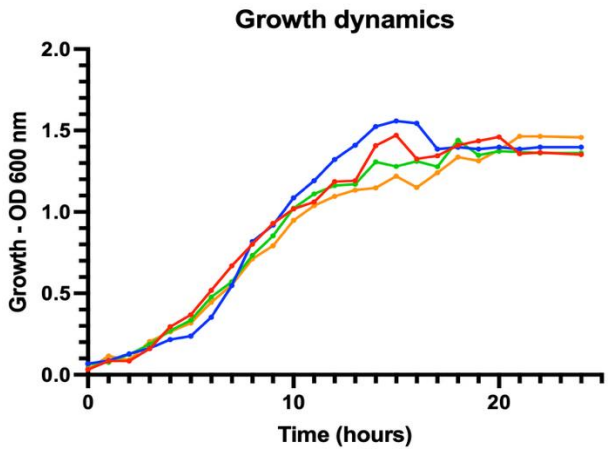

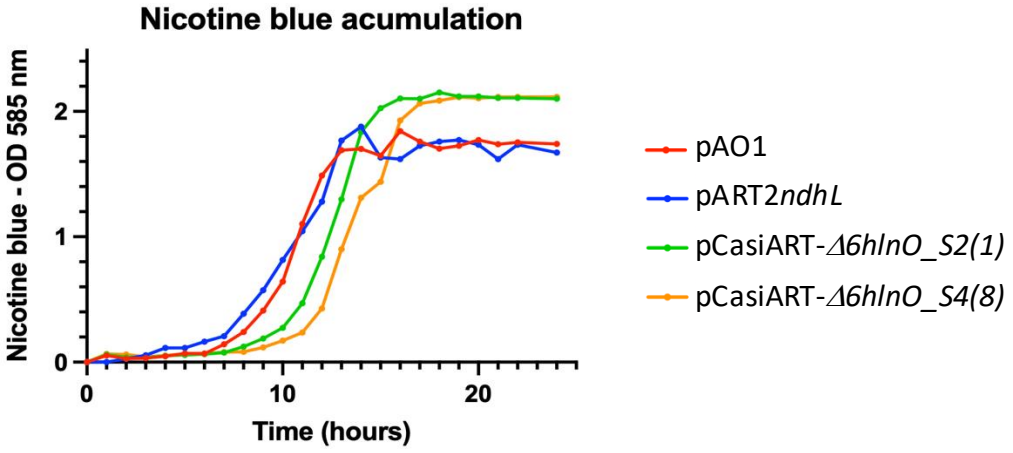

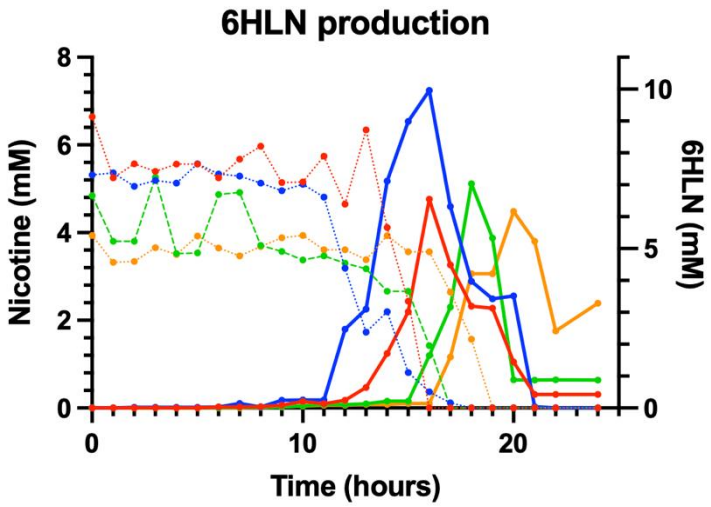

Values	pAO1	pART2*ndhL*	pCasiART-*Δ6hlnO_S2(1)*	pCasiART-*Δ6hlnO_S4(8)*
μ_max_OD 600 (μ/h⁻¹)	0.29	**0.41**	0.27	0.27
Lag_OD 600 (h⁻¹)	5.25	**3.2**	5.22	4.25
μ_max_OD 585 (μ/h⁻¹)	**0.52**	**0.50**	**0.56**	**0.66**
Lag_OD 585 (h⁻¹)	6.66	6.11	**8**	**10.02**
Nic_t½ (mM/h⁻¹)	15.50	13.87	15.42	**18.62**
6HLN Titres (g/L)	1.16	**1.77**	1.25	1.09
STY (g/L/h⁻¹)	0.068	**0.104**	0.066	0.052

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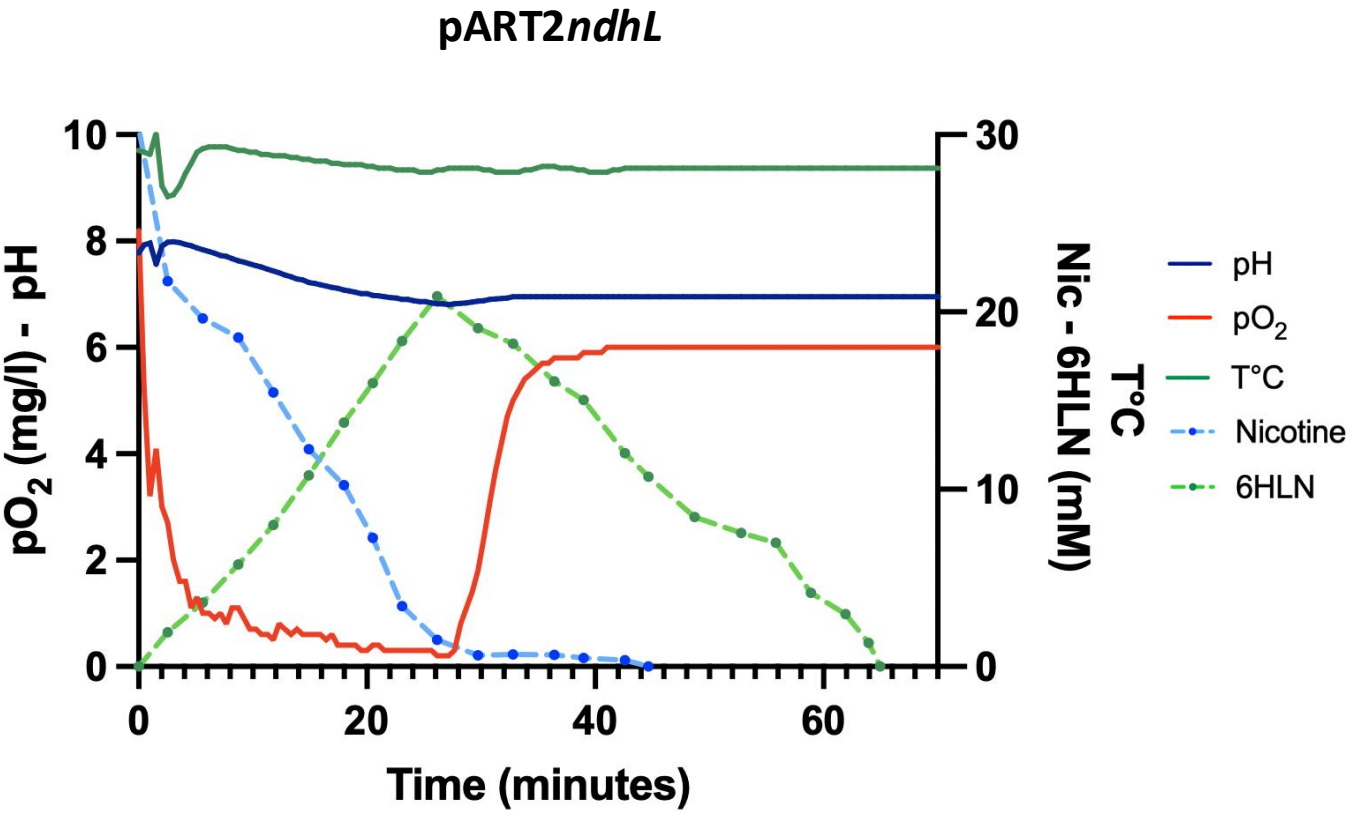

Tiberius I. M.

Summary

Biology Department, Alexandru Ioan Cuza University of Iași, Romania

Paenarthrobacter nicotinovorans **can break-down nicotine and use it for growth**

The genome of *Paenarthrobacter nicotinovorans* was sequenced *and* contains one chromosome and one plasmid

The nicotine proteome and transcriptome were solved, and we know have a better idea how nicotine catabolism is regulated and integrated into the general metabolic pathways.

We are on our way to modify the bacteria so that it will convert nicotine to useful compounds such as 6-hydroxy-L-nicotine more efficiently

Thanks

Biology Department, Alexandru Ioan Cuza University of Iași, Romania

Collaborators:

Prof. **Darie Costel, PhD**
Lead, Biochemistry & Proteomics Group
Department of Chemistry & Biomolecular Science,
Clarkson University, Potsdam, NY, USA
- nicotine induced proteome of P. nicotinovorans

apl. Prof. **Roderich Brandsch, PhD**
Institute of Biochemistry and Molecular Biology,
Freiburg i. Br., Germany
- nicotine metabolism and pAO1 molecular organization

BioActive research group

https://www.facebook.com/BioActive.bio.uaic.ro

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INOVARE SI CREATIVITATE

UNIVERSITATEA „ALEXANDRU IOAN CUZA“ din IAȘI

CommScie

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PN-III-P4-ID-PCE-2020-0656

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