Arthrobacter nicotinovorans pAO1+
Why do we need it's proteome?

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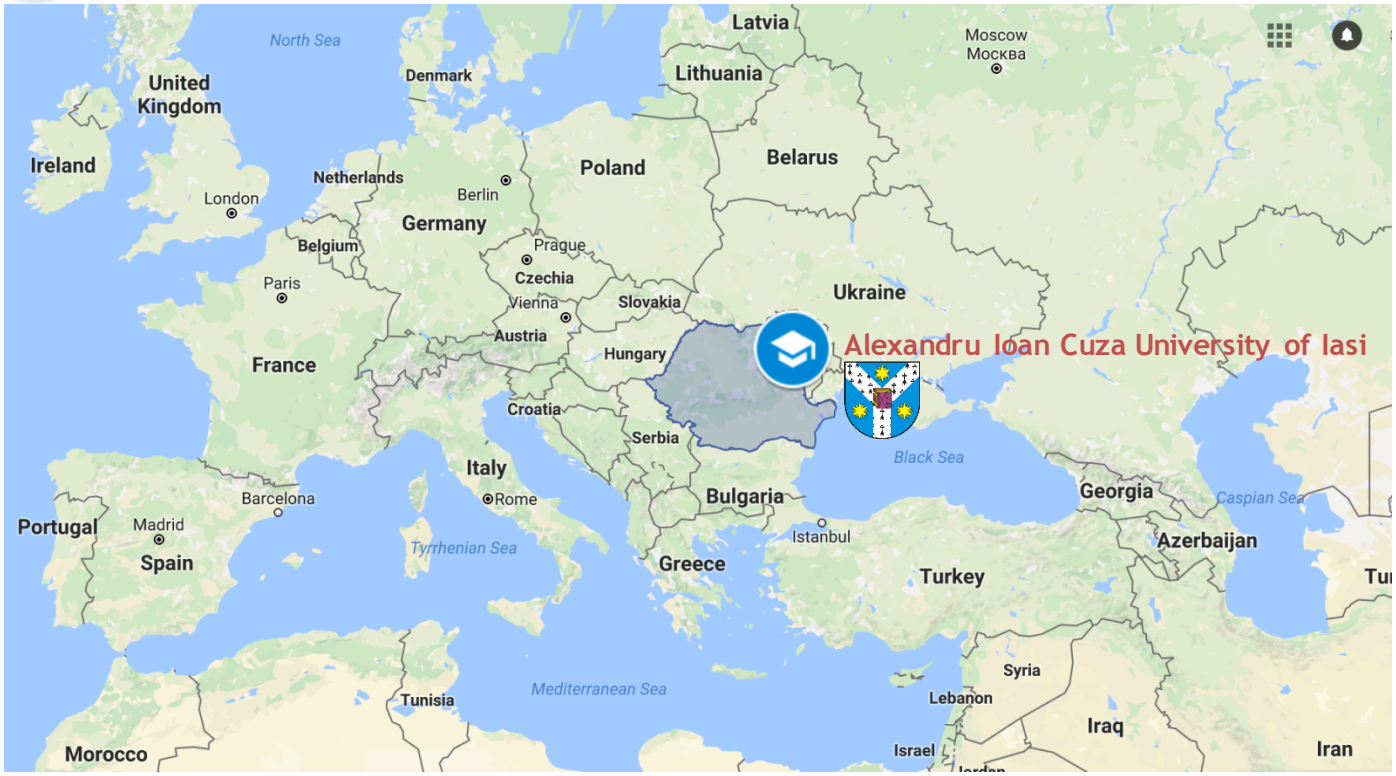
Marius Mihășan, PhD.

Faculty of Biology
Alexandru Ioan Cuza University of Iaşi, Romania

A bit about my home country

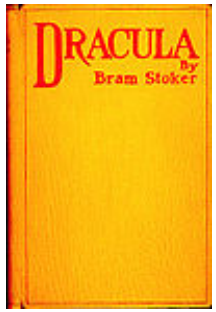


Romania

Area: 92,043 sq mi

Population: 20,121,641

Transylvania



Flight history:

Traian Vuia
the first airplane to take off on its own power

Aurel Vlaicu
flew some of the earliest successful aircrafts

Henri Coandă
discovered the Coandă effect of fluidics

Chemistry:

Lazăr Edeleanu
first to synthesize amphetamine

Costin Neniţescu
numerous new classes of inorganic compounds

Nicolae Teclu
the Teclu burner

Biology:

Victor Babeș
more than 50 types of bacteria

Emil Racoviță
founder of biospeleology

Nicolae Paulescu
discovered insulin

Emil Palade
Nobel Prize for contributions to cell biology (ribosomes)

Physics:

Ştefan Procopiu - the Bohr-Procopiu magneton

Facts and figures

- ✓ The **oldest** Romanian university
- ✓ **Diplomas recognized** all over Europe
  - ✓ **15** faculties
  - ✓ **93** Bachelor programs
  - ✓ **176** Master programs
  - ✓ **26** PhD programs

Sciences

- ✓ Biology
- ✓ Chemistry
- ✓ Computer Science
- ✓ Geography and Geology
- ✓ Mathematics
- ✓ Physics

Social Sciences & Humanities

- Economics and Business Administration
- History
- Law
- Letters
- Orthodox Theology
- Philosophy and Social – Political Sciences
- Physical Education and Sports
- Psychology and Education Sciences
- Roman – Catholic Theology
- Center for European Studies

Faculty of Biology

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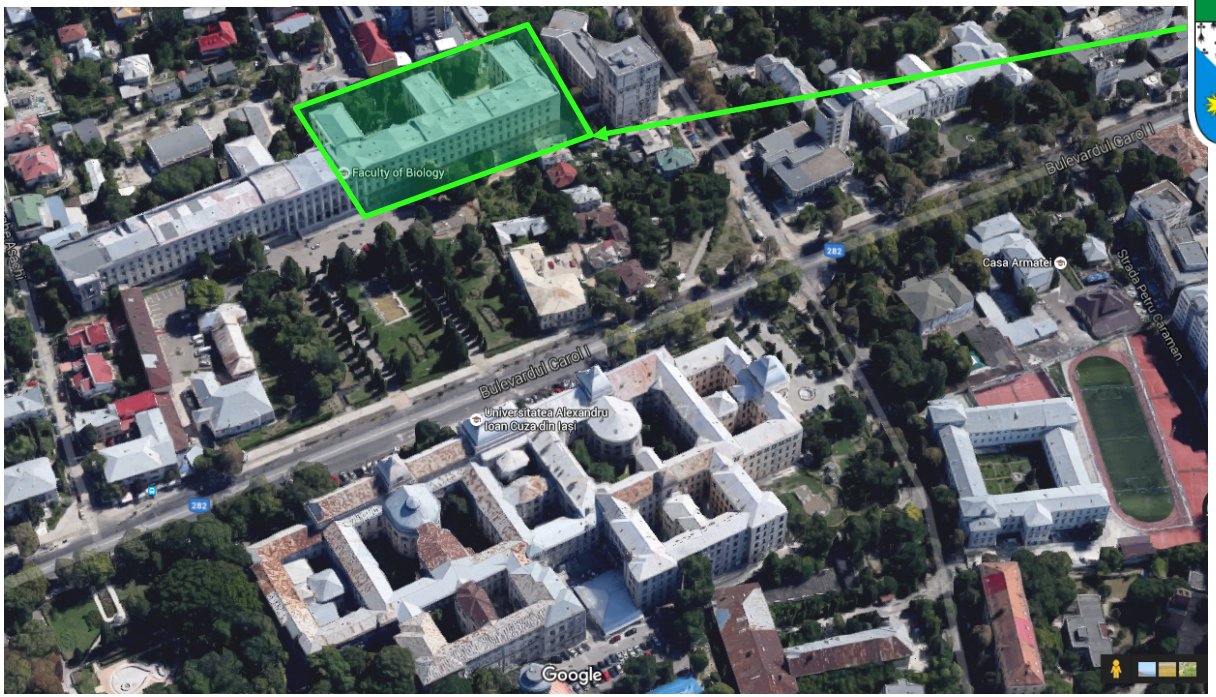


Founded in **1948**

- ✓ **758** students:
  - **20** PhD students
- ✓ **46** full-time faculty members
- ✓ **16** technicians and administrative staff

Why I am here at Clarkson?

1. identify all the nicotine-induced proteins in *Arthrobacter nicotinovorans*

2. Observe and learn the dynamics of a research group here in US
3. Attend lectures and understand the US academic system

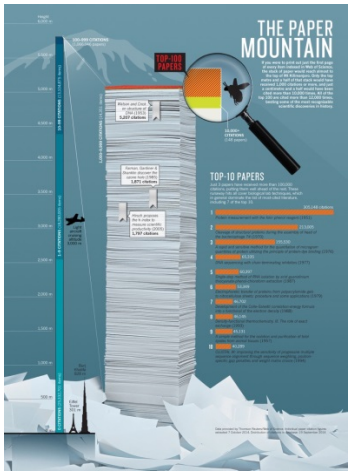
FULBRIGHT ROMANIA

Sponsor: U.S. Department of State, Romanian-U.S. Fulbright Commision
Exchange Visitor Program Number G-1-00005

Why are we <u>still</u> studying bacteria?

- restriction enzymes;
- T4 DNA ligase;
- plasmids;
- thermostable DNA polymerase;
- CRISPR/Cas9

The molecular biology revolution



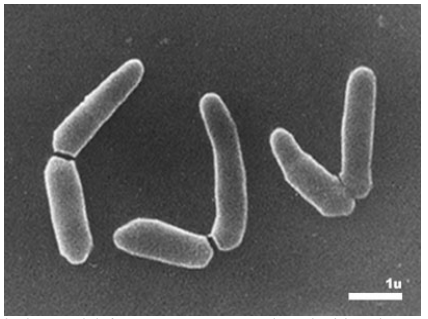
The top 100 papers of all time, 550 | NATURE | VOL 514 | 2014:
1. Lowry; 2. Laemmli; 3. Bradford;
4. Sanger 10. ClustalW;
12 and 14 BLAST; 63. PCR;

- Early diagnostic and treatment of diseases
- Forensic investigations
- Biotechnologies for the production of novel drugs and advanced materials
- Biotechnologies for high yield food and energy production

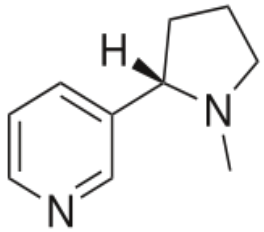
Knowledge on enzymes and metabolism of less than 2% of existing bacteria

Arthrobacter's as strains with an high biotechnological potential

- member of the *Micrococcaceae* family
- Gram-positive, obligate aerobes, non-motile
- changing shape during the life cycle from rod shaped to coccoid
- ubiquitous in polluted and toxic soil samples
- **extreamly high metabolic versatility**

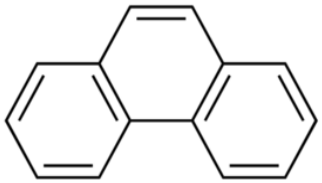


Arthrobacter globiformis IFO 12137. Irregular rods with V-shape and clubbed ends. Photo by:T. Tamura, T. Nishii & K. Hatano



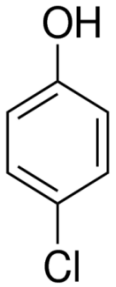
Nicotine

Arthrobacter nicotinovorans



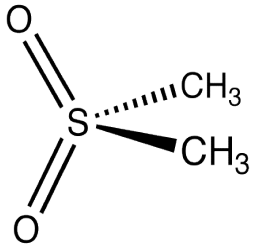
Phenantrene

Arthrobacter phenanthrenivorans



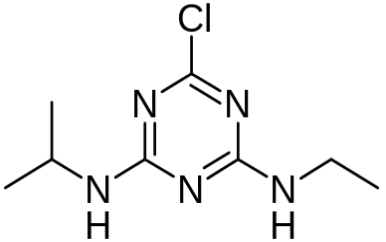
4-chlorophenol

Arthrobacter chlorophenolicus



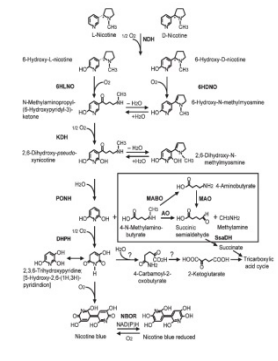
Dimethyl sulfone

Arthrobacter sulfonivorans



Atrazine

Arthrobacter sp. AK-YN10

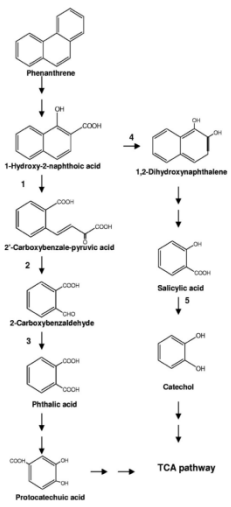


Appl Microbiol Biotechnol (2006) 69: 493–498
DOI 10.1007/s00253-005-0228-0

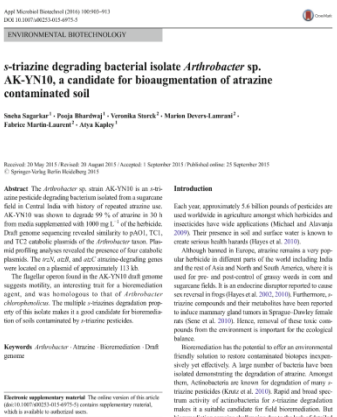
MINI-REVIEW

Roderich Brandsch

Microbiology and biochemistry of nicotine degradation



Vandera et al., Applied and Environmental Microbiology. 2012;78(3):621-627



pAO1 megaplasmid of *Arthrobacter nicotinovorans*

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Rittenberg group, USA

8 papers in JBC between 1959-1972 on the nicotine metabolism of the **P-34** **Gram-negative** strain isolated from soil

Same bacteria

Decker group, Germany

6 papers in various journals between 1961-1969 on a highly similar nicotine metabolism in ***Arthrobacter oxydans***

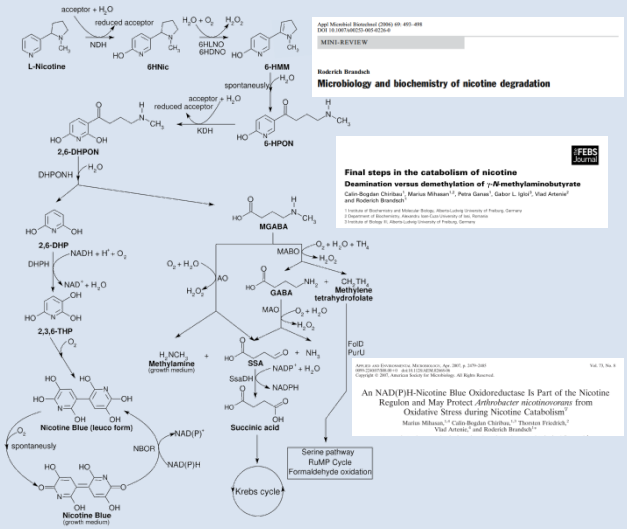
since 1990

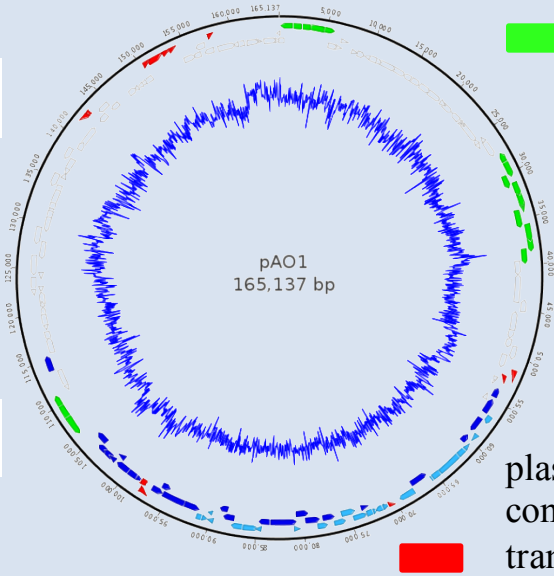
Brandsch group, Germany

isolation and sequencing of the **p**lasmid **A**rthrobacter **O**xydans 1 – **pAO1;** molecular biology of the nicotine degradation pathway.

A. oxydans reclassified as ***A. nicotinovorans*** (Kodama et al. 1992) and as ***Paenarthrobacter nicotinovorans*** (Busse H. 2016)

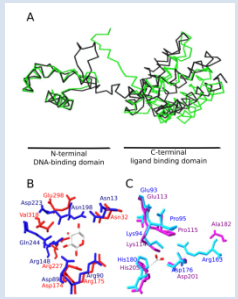
Nicotine metabolism





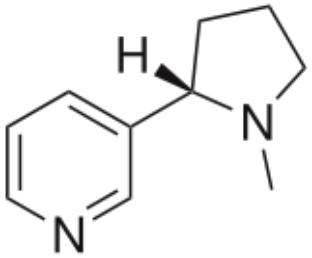
Carbohydrate catabolism

3 gene clusters, the largest one – degradation of xylose



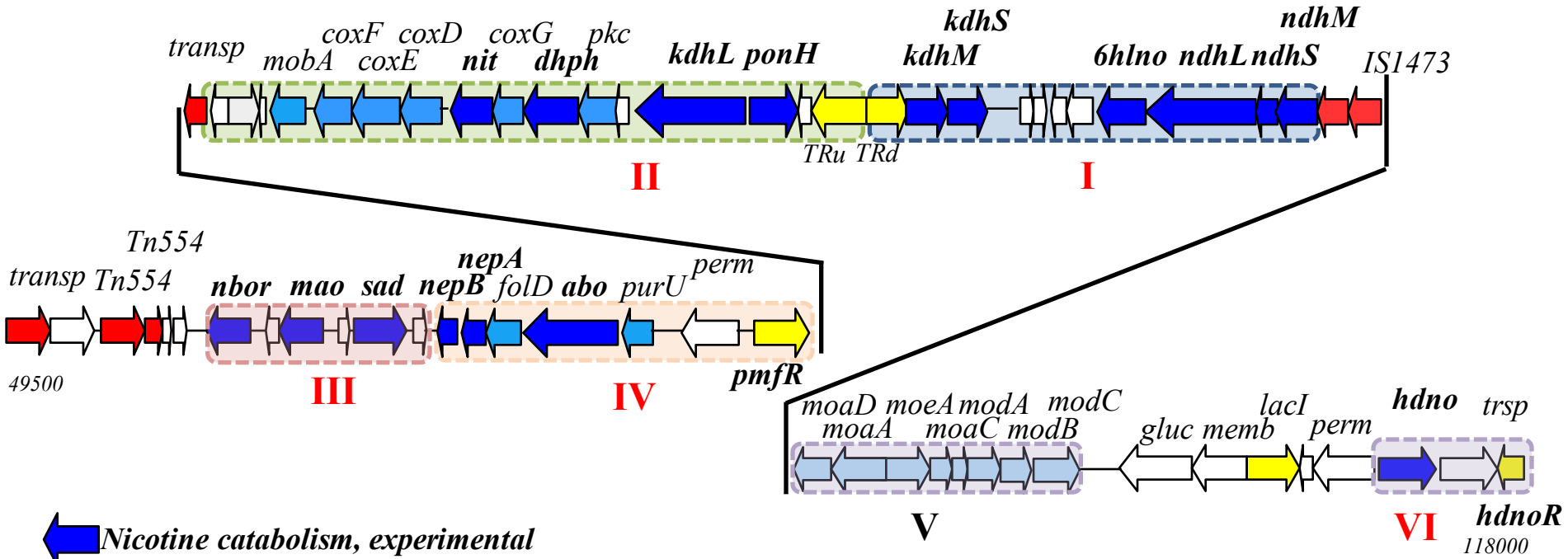
plasmid replication and partition;
conjugation;
transposons and insertion elements

The *nic* gene cluster of *Arthrobacter nicotinovorans* pAO1



Bacterial nicotine catabolism:

- **the pyridine pathway** – *Arthrobacter* and *Nocardioides* (Brandsch 2006)
- the pyrrolidine pathway - *Pseudomonas* (Tang et al. 2013)
- the VPP (**v**ariant of **p**yridine and **p**yrrolidine) pathway (Yu et al. 2015)



Nicotine catabolism, experimental

Nicotine catabolism, putative

Transcription factors, ***experimental*** *or putative*

Putative ORFs, no function

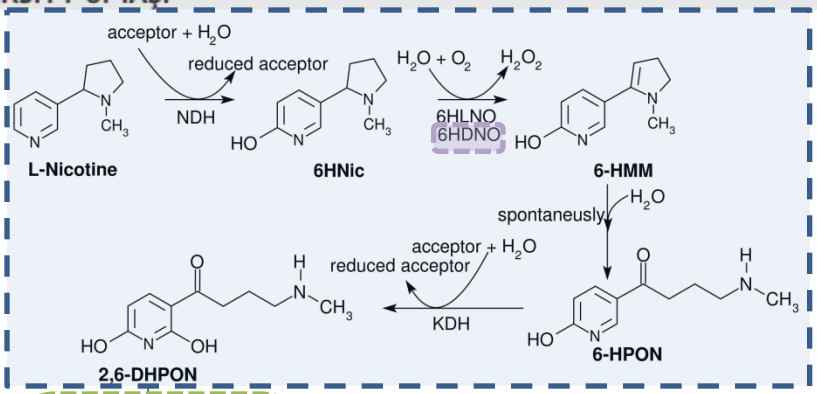
Transposons and insertion elements

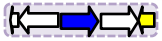
The nicotine catabolic pathway in *A. nicotinovorans*

I 

NDH, KDH - heterotrimeric $(\alpha\beta\gamma)_2$ FAD, **MCD** and FeS clusters

NDH acts on both L- and D-nicotine;

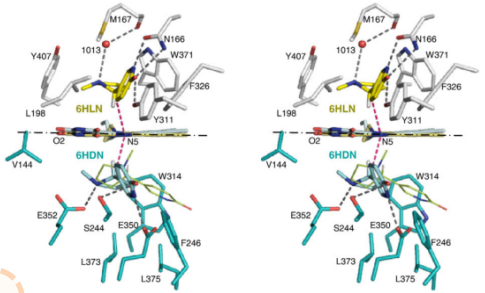


VI 

6HLNO and **6HDNO**

absolute stereospecificity,

genetically and structurally unrelated flavoenzymes

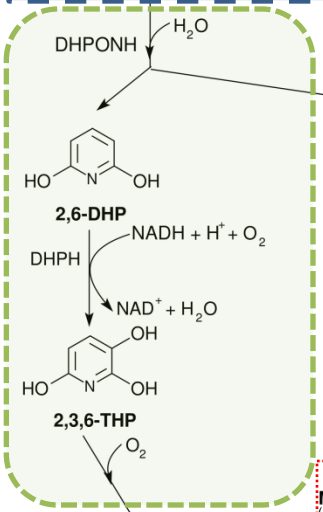


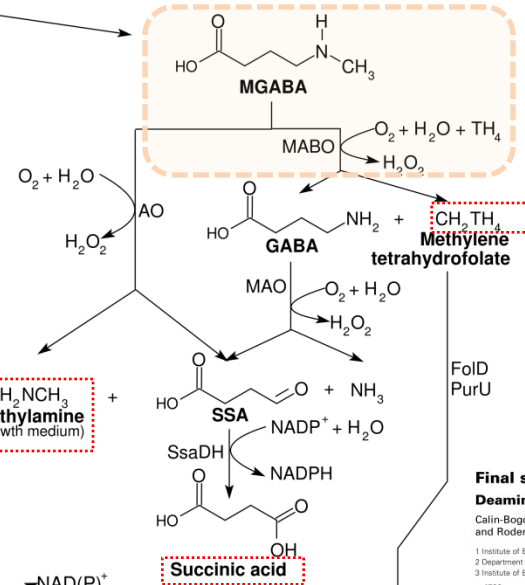
Kachalova et al., 2010, J Molec Biol., 396(3):785-799

II 

DHPONH - α/ß fold C-C bond hydrolase

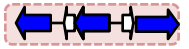
Schleberger et al., 2006, J Molec Biol., 367(2):409-418

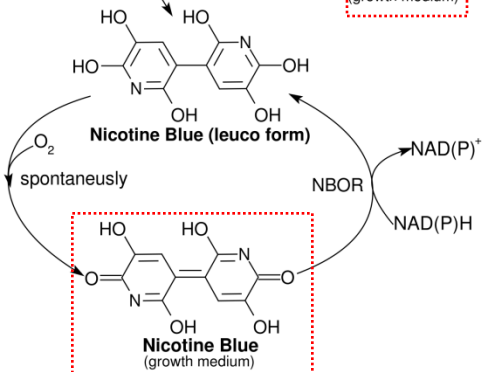


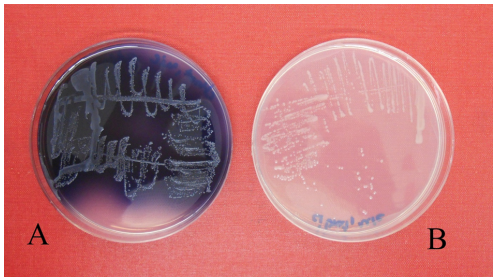


IV 

Gene regulation probably switches between the two variants

III 





Appl Environ Microbiol. 2007 Apr; 73(8): 2479–2485.
Published online 2007 Feb 9. doi: 10.1128/AEM.02668-06
PMCID: PMC1855579

An NAD(P)H-Nicotine Blue Oxidoreductase Is Part of the Nicotine Regulon and May Protect *Arthrobacter nicotinovorans* from Oxidative Stress during Nicotine Catabolism

Marius Mihasan,[1,4] Calin-Bogdan Chiribau,[1,3] Thorsten Friedrich,[2] Vlad Artenie,[4] and Roderich Brandsch[1,*]

Final steps in the catabolism of nicotine

Deamination versus demethylation of γ-*N*-methylaminobutyrate

Calin-Bogdan Chiribau[1], Marius Mihasan[1,2], Petra Ganas[1], Gabor L. Igloi[3], Vlad Artenie[2] and Roderich Brandsch[1]

1 Institute of Biochemistry and Molecular Biology, Alberts-Ludwig University of Freiburg, Germany
2 Department of Biochemistry, Alexandru Ioan-Cuza University of Iasi, Romania
3 Institute of Biology III, Alberts-Ludwig University of Freiburg, Germany

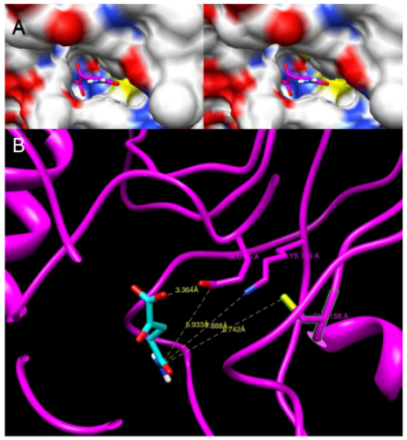
1528

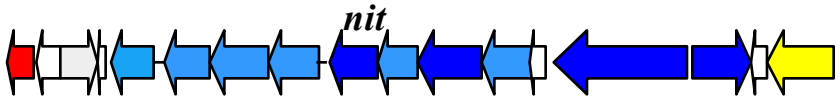
FEBS Journal 273 (2006) 1528–1536 © 2006 The Authors Journal compilation © 2006 FEBS

Blank spots in nicotine metabolic pathway *in A. nicotinovorans*

1. Is Nicotine-Blue (NB) the final product of the pathway?

A. a ω-amidase for α-ketoglutaramate is part of the *nic*-gene cluster

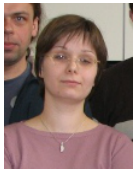


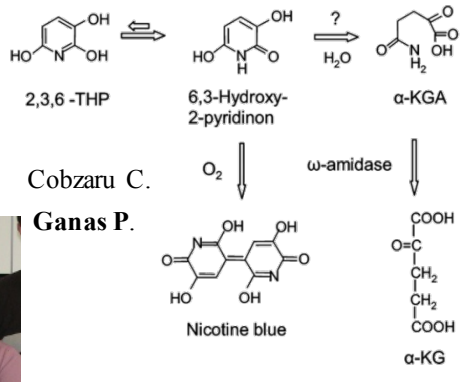


Flexible targeted docking

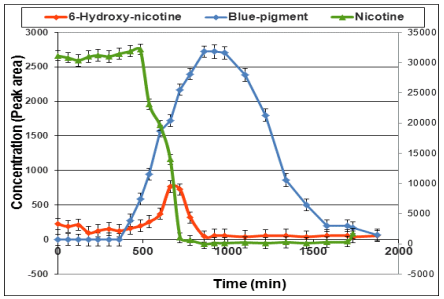
α-keto- glutaramate can be docked into the NIT active site

Cobzaru, C., et al. (2011) *Research in Microbiology*, *162*(3), 285-91.





B. Nicotine-blue is consumed in old cultures

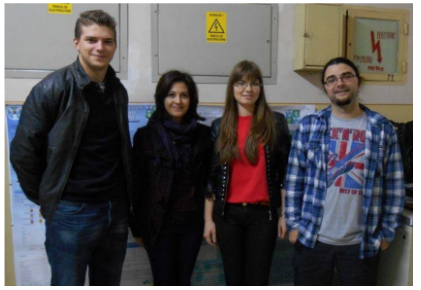


C. gross energy output is low - 7.5 ATP's and one oxaloacetate / one nicotine

A pyridine ring cleaving enzyme must exist

- 2,3,6-THP – opened by a hypothetical cyclase in *Rhodococcus rhodochrous* PY11
- 2,5-DHP – opened by a dioxygenase in *P. putida S16* and *Ochrobactrum sp.* SJY1

Unknown enzyme? Chromosomal gene?



Boiangiu R., B.Sc **Doina Guzun., B.Sc**
Andrei Andreea., M.Sc

Blank spots in nicotine metabolic pathway *in A. nicotinovorans*

2. Which are the proteins involved in nicotine transport?

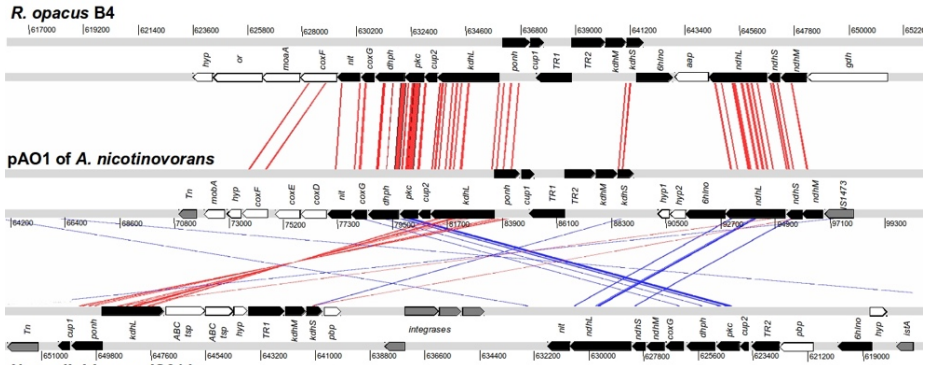
L-nicotine uptake in *A. nicotinovorans*:
- **Dependent** on nicotine degradation but **not on pAO1**
- **Energy-independent**
- **Saturable** with a K(m) of 6.2 microM and a V(max) of 0.70 micromol/ min/mg protein)

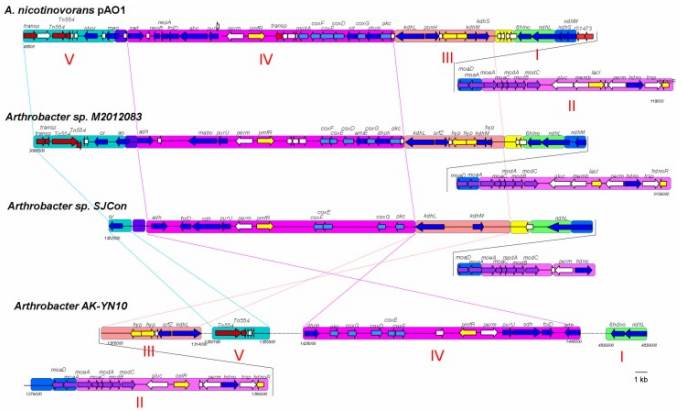
Ganas & Brandsch, 2009, *Microbiology*, *155*: 1866–77

=>

facilitated diffusion
driven by the nicotine gradient due to its intracellular degradation
by a unknown
chromosomal permease

3. Several other bacteria have the nic gene cluster, but not a functional nicotine catabolism





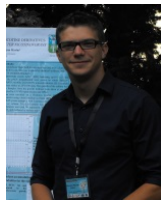
J Mol Evol
DOI 10.1007/s00239-013-9576-x

ORIGINAL ARTICLE

pAO1 of *Arthrobacter nicotinovorans* and the Spread of Catabolic Traits by Horizontal Gene Transfer in Gram-Positive Soil Bacteria

Marius Mihasan · Roderich Brandsch

Baumont V., M.Sc. France
AK-YN10 contains a plasmid



Boiangiu R., M.Sc
nic-genes in AK-YN10 are on the plasmid

Some chromosomal genes must be directly/indirectly involved in nic catabolism

Potential applications of the *nic*-pathway

1. Decontamination of nicotine-containing waste, soil and water from tobacco industry

3,00,274 tones of nicotine-containing waste/year, 18 g nicotine/per kg

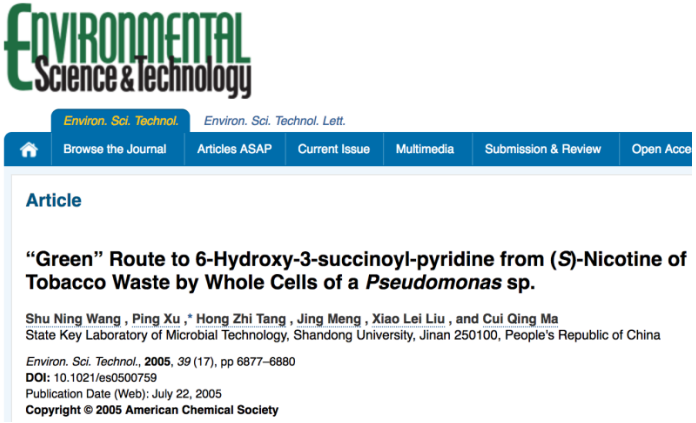
- According to EPA the waste is **Toxic Release Inventory (TRI)** chemicals
- According to the European Union Regulations (EUR) - **"toxic and hazardous"** waste (when the concentration of nicotine exceeds 0.05% w/w)

2. Engineering the *nic*-pathway for the production of value-added chemicals

Sci Rep. 2015; 5: 16411. PMCID: PMC4647180
Published online 2015 Nov 17. doi: 10.1038/srep16411

Sustainable production of valuable compound 3-succinoyl-pyridine by genetically engineering *Pseudomonas putida* using the tobacco waste

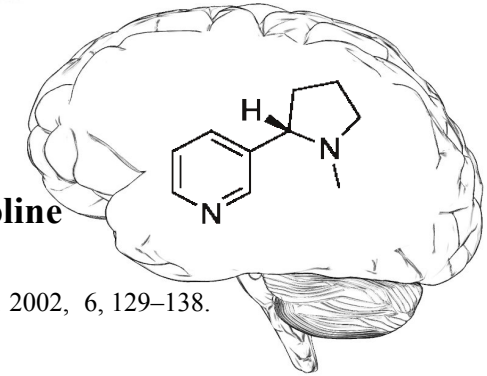
Weiwei Wang,[1] Ping Xu,[1] and Hongzhi Tang[a,1,*]



6-hydroxy-L-nicotine – a novel neuroprotective agent?

- anti-oxidant effects at low concentrations
- cognition-enhancing agent
- well-known agonist **of nicotinic acetylcholine receptors (nAChR)**

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



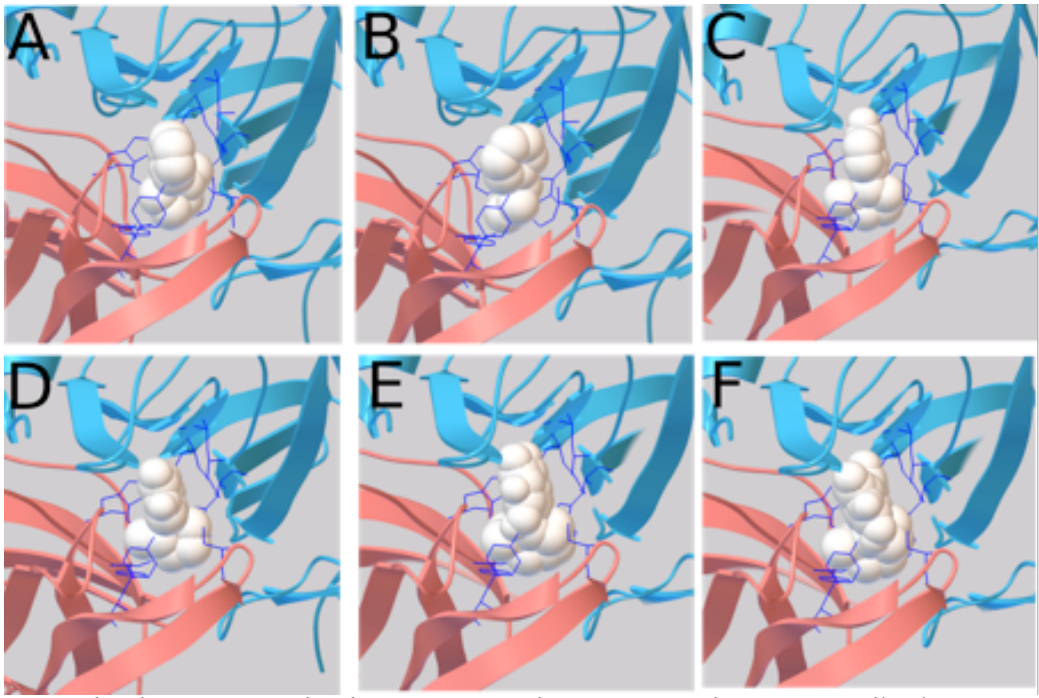
- short half-time (about 2 hours)
- proven negative effects on various organs such as lungs
- linked to cigarettes and the negative publicity associated with smoking

Beccafusco, *Mol Interv*. 2004 Oct;4(5):285-95.

Could nicotine derivatives from *Arthrobacter* bind nAChR's?

In silico - Targeted docking

– *rigid receptor* subunits A and B from PDB ID 1UW6
- *targeted region* - a cube of aprox. 150 Å^3 centered on Tyr143 from subunit A
- *flexibile ligands* – 3D structures obtained from PubChem database

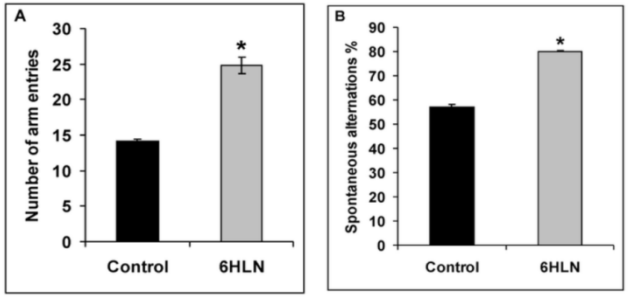


A - L- Nicotine, **B** - D-Nicotine, **C** - 6HLNic, **D** - 6HDNic, **E** - 2,6-Dihydroxy-N-methylmyosmine, **F** - 2,6- Dihydroxypyridine

6-hydroxy-L-nicotine – a novel neuroprotective agent?

Animals tests

- male Wistar rats (3-4 months old)
- 6-hidroxy-L-nicotine was injected intraperitonealy, 0.3 mg/kg b.w, daily, for 7 consecutive days.



Y-maze task

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

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Original article

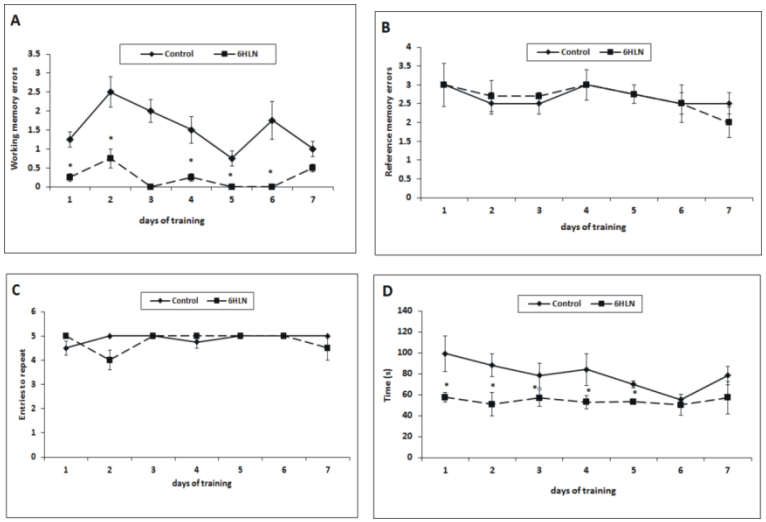
Nicotine versus 6-hydroxy-L-nicotine against chlorisondamine induced memory impairment and oxidative stress in the rat hippocampus

Lucian Hritcu*, Radu Ionita, Diana Elena Motei, Cornelia Babii, Marius Stefan, Marius Mihasan*

Department of Biology, Alexandru Ioan Cuza University of Iasi, Bd. Carol I, No. 11, 700506, Romania

Radial arm-maze task

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



Neuroscience Letters 591 (2015) 41–47

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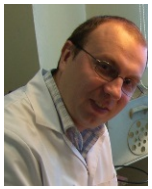
journal homepage: www.elsevier.com/locate/neulet

Research article

Enhanced behavioral response by decreasing brain oxidative stress to 6-hydroxy-L-nicotine in Alzheimer's disease rat model

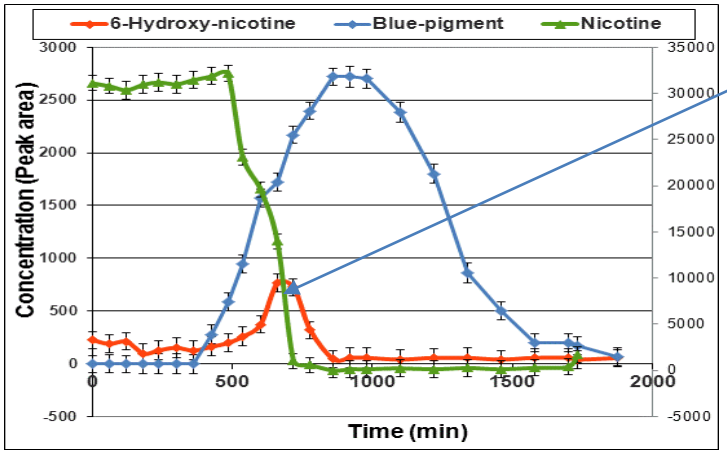
Lucian Hritcu[a,*], Marius Stefan[a], Roderich Brandsch[b], Marius Mihasan[a]



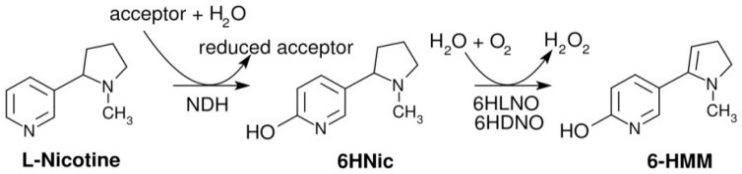
Prof. **Hritcu L.**, PhD

Ioniță R., PhD student

Steps towards an *Arthrobacter nicotinovorans* based biotechnology for the production of 6HNic



6-HNic accumulates in the medium for a short period of time



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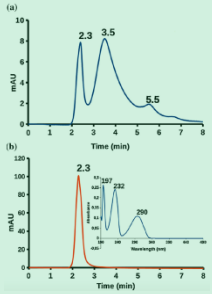
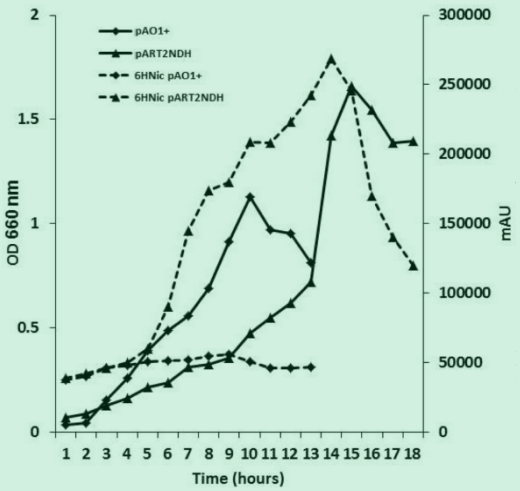
Prof. Stefan M., PhD

Babii C., PhD student Mihalache G., PhD student

Moței E.D., B.Sc.





$ZnSO_4$ (mM)	Nicotine consumption rate (mmoles/h)	6HNic maximal level (mM)
0	0.2001	0.95
0.05	**0.5256**	1.53
0.1	0.4670	**1.74**
0.15	0.4601	1.01
0.2	0.2527	0.37
0.3	0.2707	0.35
0.5	0.2651	0.34
1	0.1672	0.24

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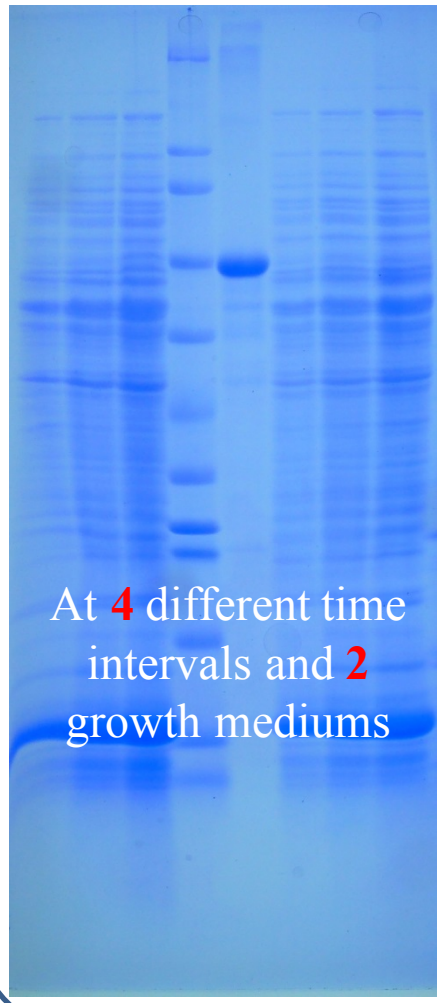
Expected impact of the MS/MS approach



AJ507836.1 (pAO1 sequence)

and

BDDW01000000 (draft genome)

1. Identify all **nicotine-induced proteins** encoded by pAO1 genes
2. Focus on **transcription factors and transporters** related no nicotine catabolism (plamidial or chromosomal)
3. Identify some candidates for the **pyridine ring cleaving enzyme**
4. Are any chromosomal gene products involved in nicotine metabolism and if yes, how?

Thanks

Collaborators:

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

Prof. **Vlad Artenie, PhD**
Biology Faculty, A.I. Cuza University of Iasi
- fruitful talks on enzyme assays and oxidative stress

Prof. **Zenovia Olteanu, PhD**
Biology Faculty, A.I. Cuza University of Iasi
- ***coordinator for one of the PostDoc projects***
- ***help with the administrative and other issues related to the academic life at UAIC***

Prof. **Lucian Hritcu**, PhD
-6HNic testing on lab rats
-manuscript writing and publication

Prof. **Marius Ştefan**, PhD
-microbial physiology and taxonomy
-research projects writing and management

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Questions?