

# The *Arthrobacter* genus *nic*-gene clusters share a modular design



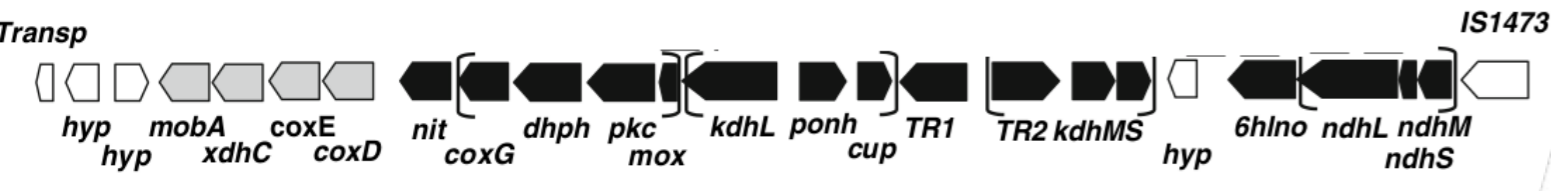
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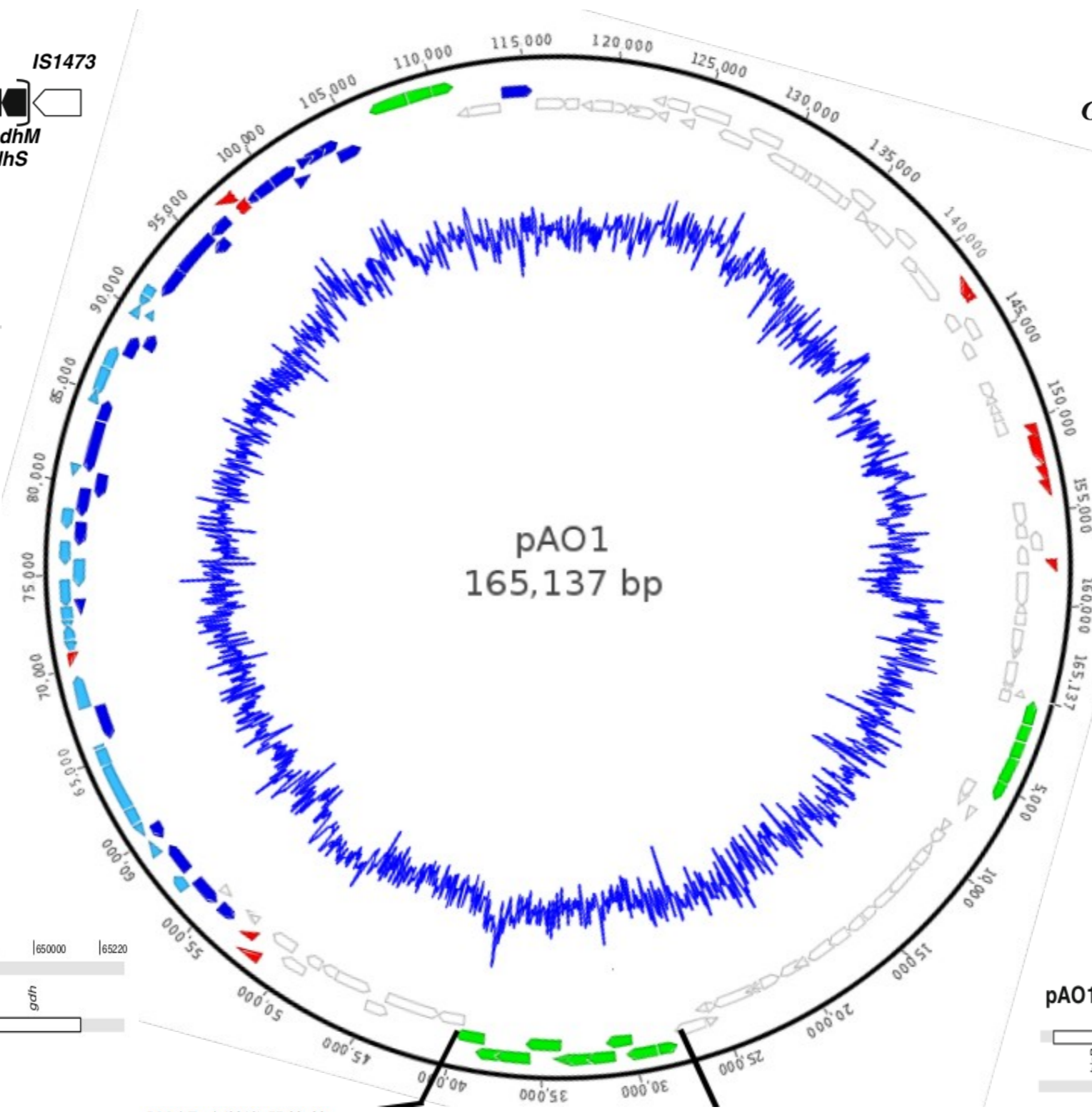
# Nicotine-metabolism



Appl Microbiol Biotechnol (2006) 69: 493–498  
DOI 10.1007/s00253-005-0226-0

MINI-REVIEW

Roderich Brandsch  
**Microbiology and biochemistry of nicotine degradation**

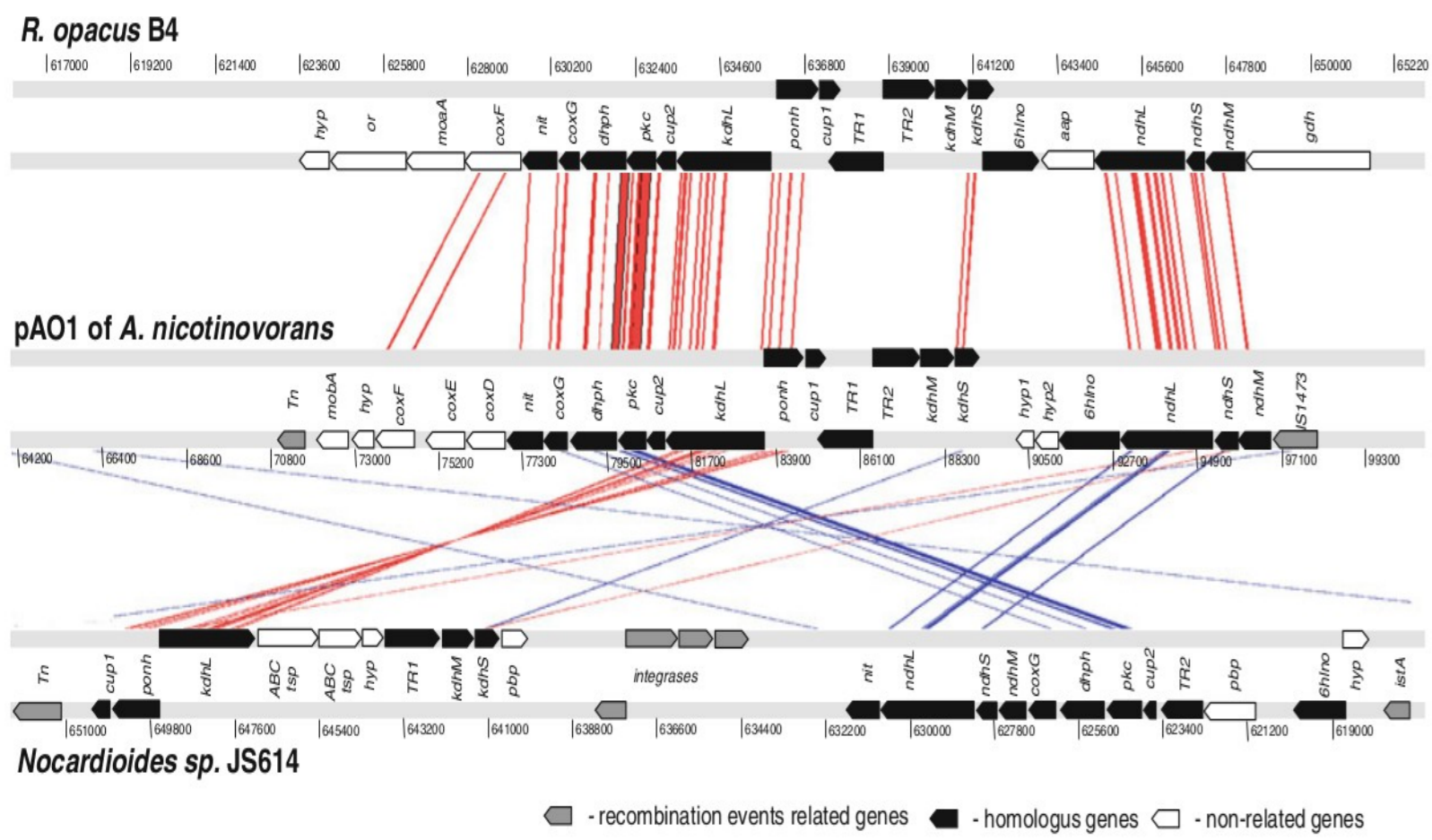


J Mol Evol (2013) 77:22–30  
DOI 10.1007/s00239-013-9576-x

ORIGINAL ARTICLE

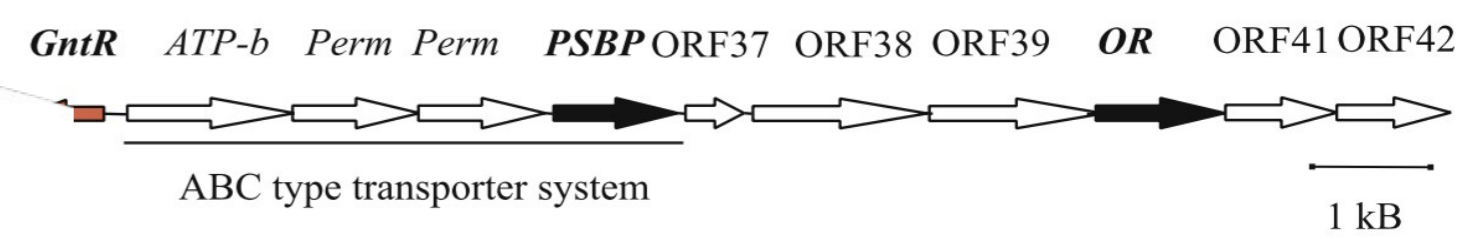
**pAO1 of *Arthrobacter nicotinovorans* and the Spread of Catabolic Traits by Horizontal Gene Transfer in Gram-Positive Soil Bacteria**

Marius Mihasan · Roderich Brandsch

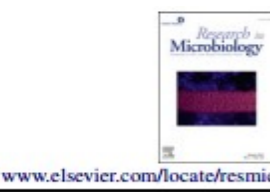


◻ - recombination events related genes ◼ - homologous genes ◻ - non-related genes

# Xylose-metabolism

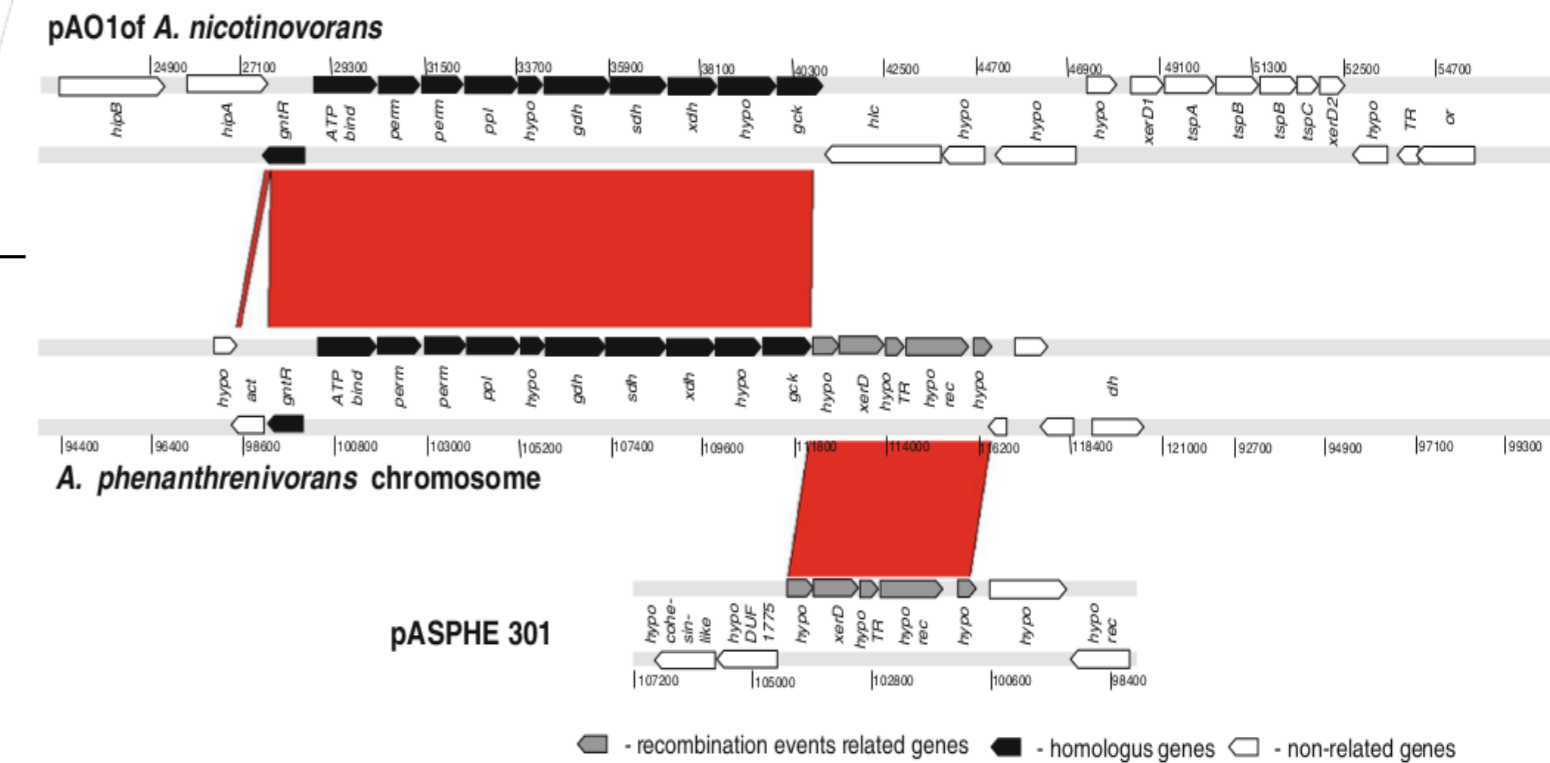


Institut Pasteur  
Research in Microbiology 164 (2013) 22–30



Evidence of a plasmid-encoded oxidative xylose-catabolic pathway in *Arthrobacter nicotinovorans* pAO1

Marius Mihasan <sup>a,\*</sup>, Marius Stefan <sup>a,1</sup>, Lucian Hritcu <sup>a,1</sup>, Vlad Artenie <sup>a</sup>, Roderich Brandsch <sup>b</sup>



◻ - recombination events related genes ◼ - homologous genes ◻ - non-related genes



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*Arthrobacter nicotinovorans* and pAO1

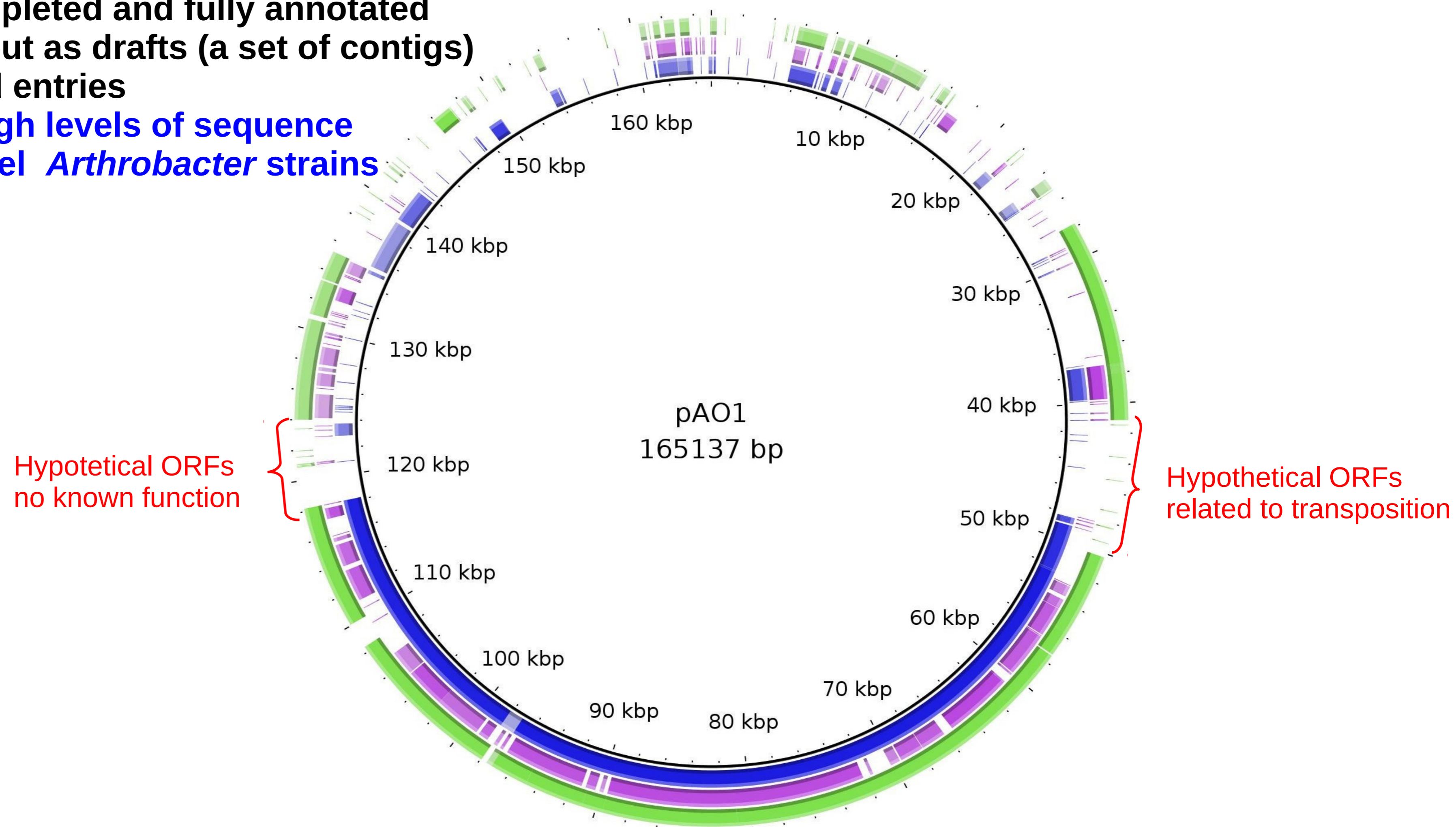
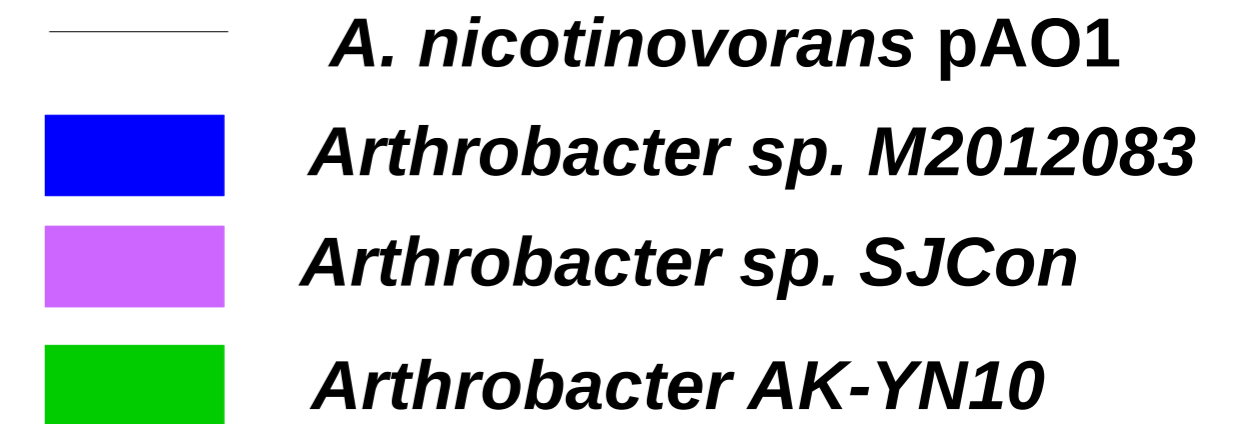
# pAO1 shares most of its *nic*-genes with other *Arthrobacter* genomes



25 entries in GenBank for *Arthrobacter* plasmids  
similarities between pAO1 and other  
*Arthrobacter* plasmids are rather low

37 entries in GenBank for *Arthrobacter* genomes:  
- 5 genomes are completed and fully annotated  
- 10 are completed, but as drafts (a set of contigs)  
- 22 are uncompleted entries

pAO1 shows high levels of sequence  
similarity with 3 novel *Arthrobacter* strains





## Draft genomes

*Arthrobacter* sp. M2012083 (GI:NZ\_AKKK000000000)  
*Arthrobacter* sp. SJCon (GI: NZ\_AOFD000000000)  
*Arthrobacter* sp. AK-YN10 (GI: NZ\_AVPD000000000)

## Reference genomes

*Arthrobacter aurescens* TC1 (GI:NC\_008711),  
*Arthrobacter chlorophenolicus* A6 (GI:NC\_011886),  
*Arthrobacter arilaitensis* Re117 (GI:NC\_014550)  
*Arthrobacter phenanthrenivorans* Sphe3 (GI:NC\_015145)  
*Arthrobacter nitroguajacolicus* Rue61a (GI:NC\_018531)

Contig assembly using MAUVE

Min LCB weight: 200  
Scoring Matrix: HOXD

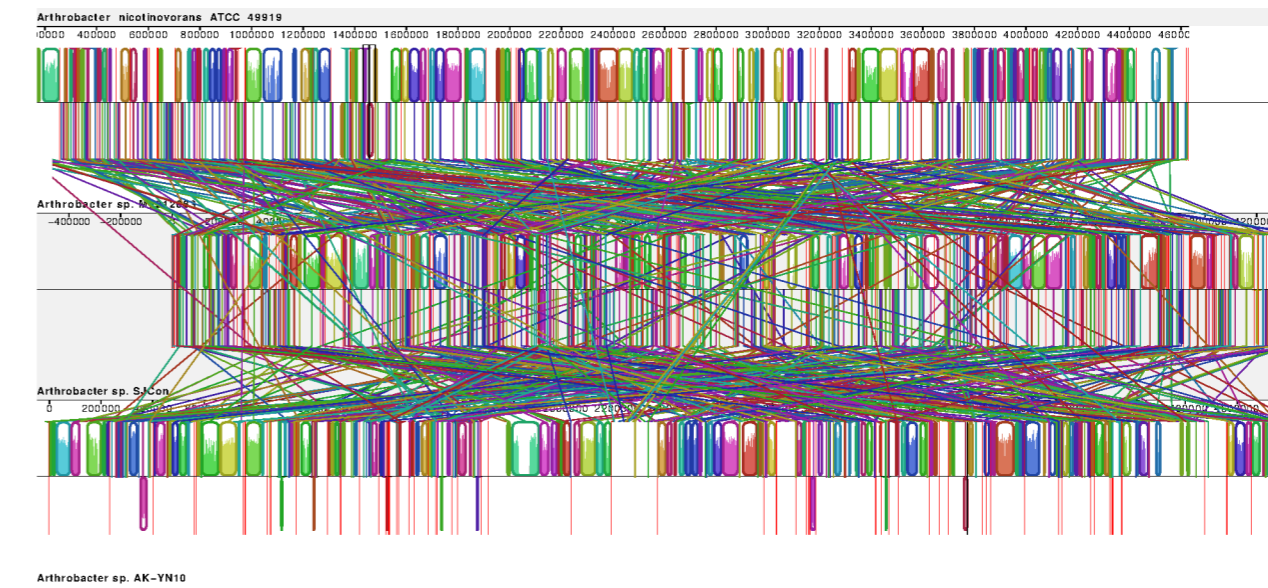
Genome annotation using RAST

v 2.0

**Final genomes**

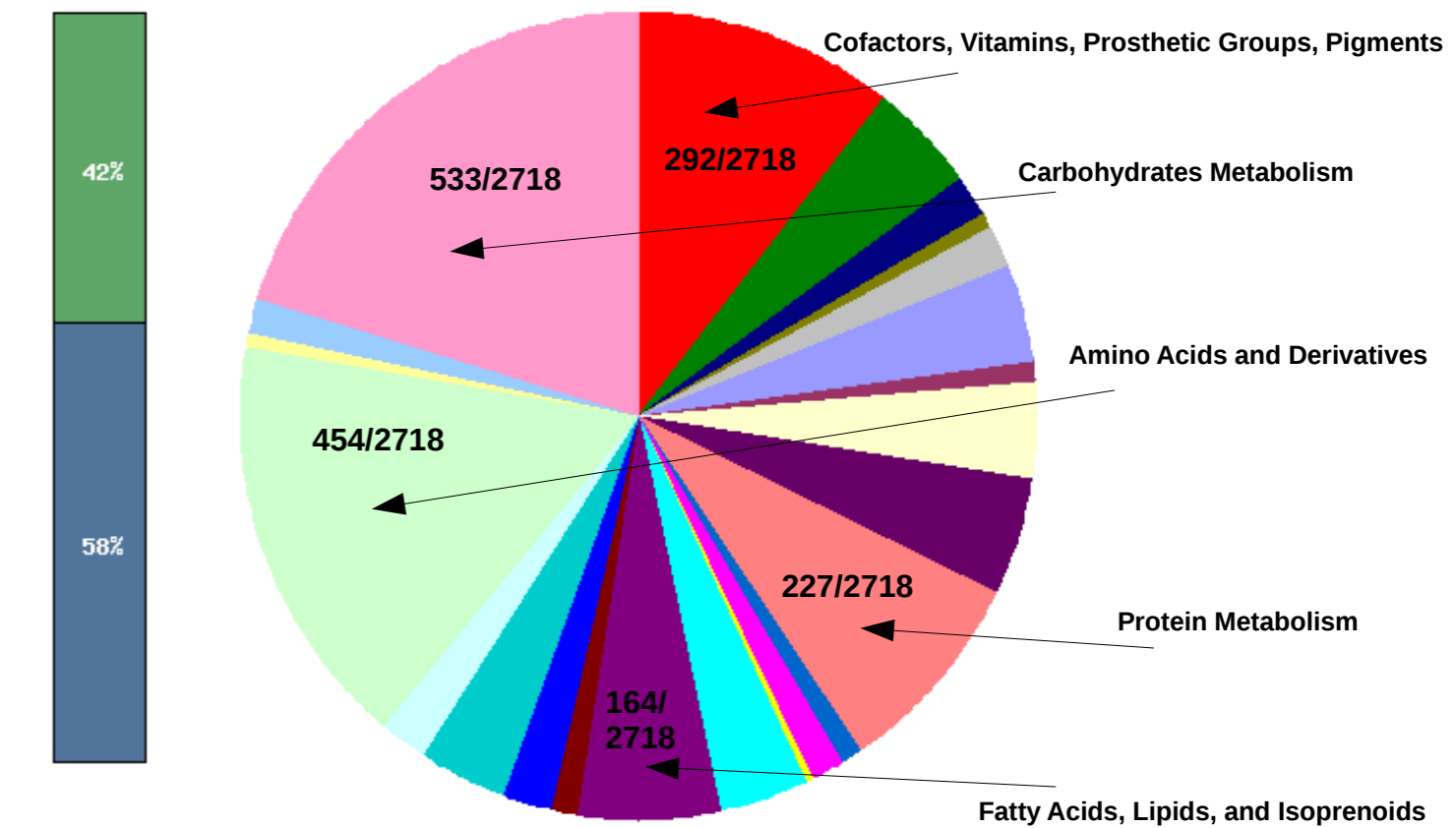
Gene collinearity  
MAUVE

Sequence similarity  
BLAST, BRIG

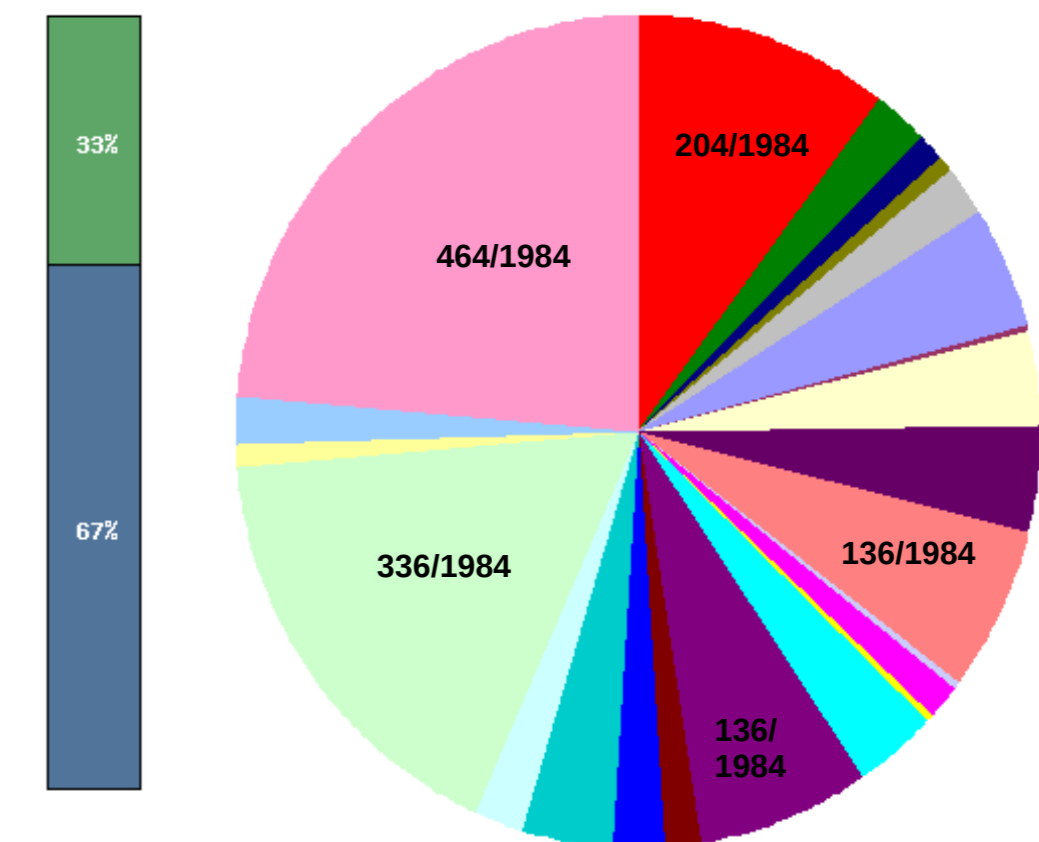


# General statistical data and metabolic profiles of the annotated *Arthrobacter* genomes

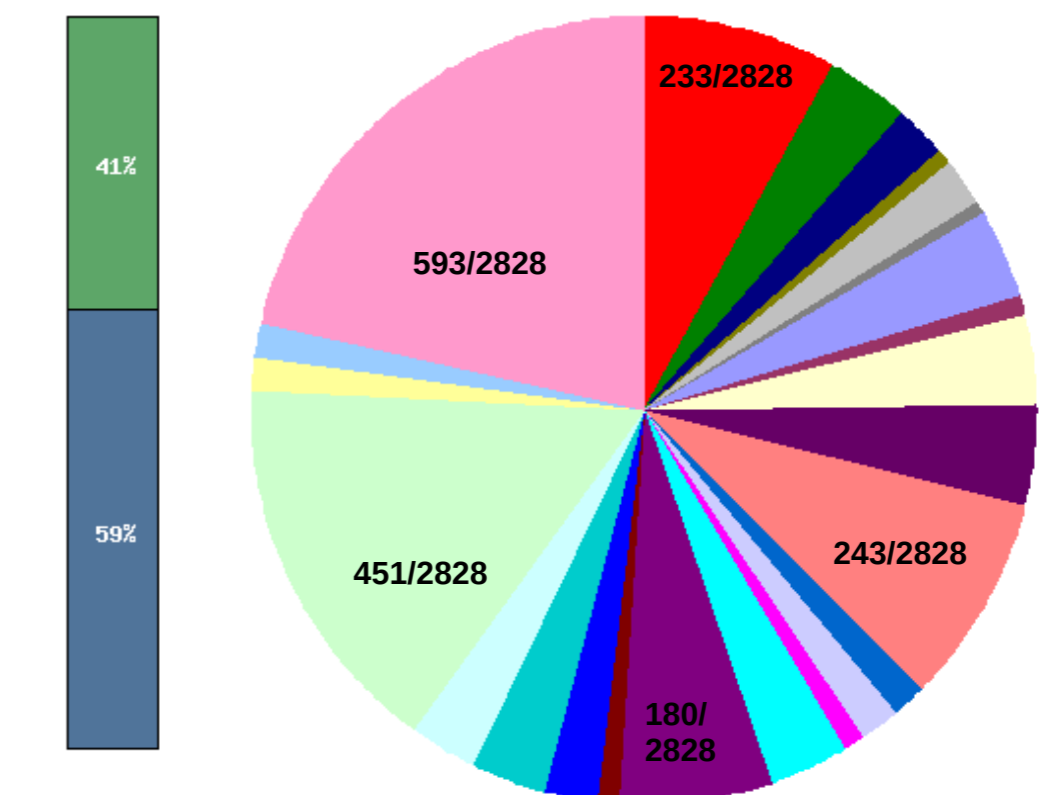
## *Arthrobacter* sp. M2012083



## *Arthrobacter* sp. SJCon



## *Arthrobacter* AK-YN10



Assembled genomes	<i>Arthrobacter</i> sp. M2012083			<i>Arthrobacter</i> sp. SJConn		<i>Arthrobacter</i> sp. AK-YN10	
Reference genomes	<i>Arthrobacter aurescens</i> TC1	<i>Arthrobacter nitroguajacolicus</i> Rue61a	<i>Arthrobacter chlorophenolicus</i> A6	<i>Arthrobacter phenanthrenivorans</i> Sphe3	<i>Arthrobacter chlorophenolicus</i> A6	<i>Arthrobacter aurescens</i> TC1	<i>Arthrobacter nitroguajacolicus</i> Rue61a
Number of Contigs:	67			142		107	
Number of assembly bases:	4629172			4389620		4839751	
Number of reference bases:	4597686	4736495	4395537	4250414	4395537	4597686	4736495
Number of LCBs:	37	53	257	134	79	98	105
Number of Blocks:	89	100	285	222	173	135	142
Breakpoint Distance:	89	100	285	222	173	135	142
DCJ Distance:	81	91	276	213	164	126	134
SCJ Distance:	178	200	570	444	346	270	284
Number of SNPs:	437118	441536	601223	534528	531846	635011	642135
Number of Gaps in Reference:	3707	3793	8645	6602	6798	6689	6937
Number of Gaps in Assembly:	3583	3643	9053	7788	8342	7207	7349
Total bases missed in reference:	806998	846192	1831672	1132545	1310535	1408168	1523527
Percent bases missed:	17.55%	17.87%	41.67%	26.65%	29.82%	30.63%	32.17%
Total bases extra in assembly:	601474	607774	2003703	1032185	1165005	1230027	1244537
Percent bases extra:	12.99%	13.13%	43.28%	23.51%	26.54%	25.42%	25.71%
Number of extra contigs:	8	8	13	40	37	56	58
Number of Inter-LCB Boundaries:	6	11	25	14	9	13	9
Contig N50:	129641	129641	129641	61782	61782	131717	131717
Contig N90:	33265	33265	33625	18136	18136	28762	28762

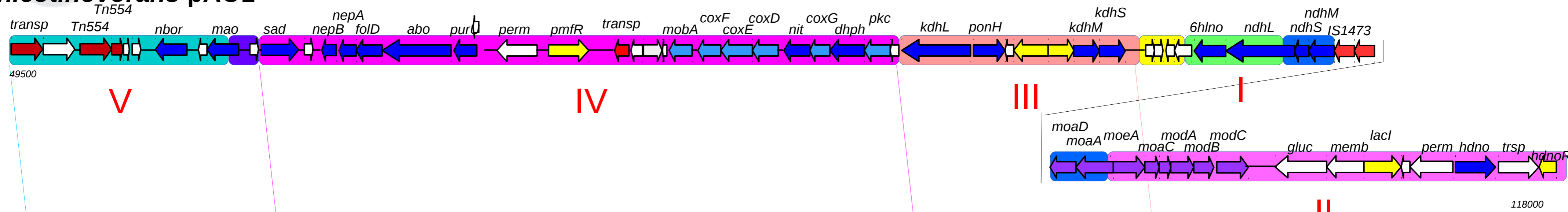
# Nic-gene cluster collinearity analysis among 3 *Arthrobacter* genomes



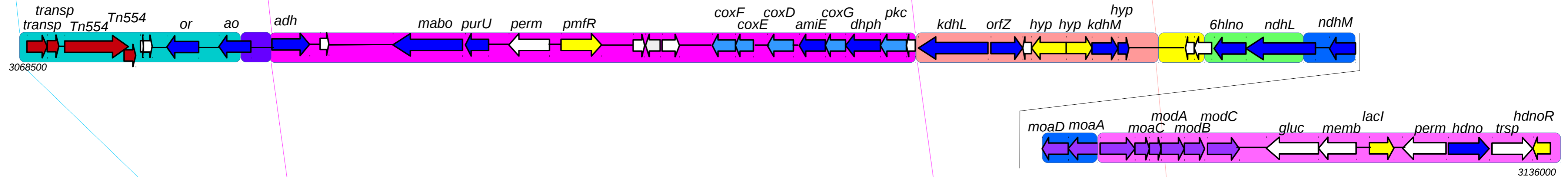
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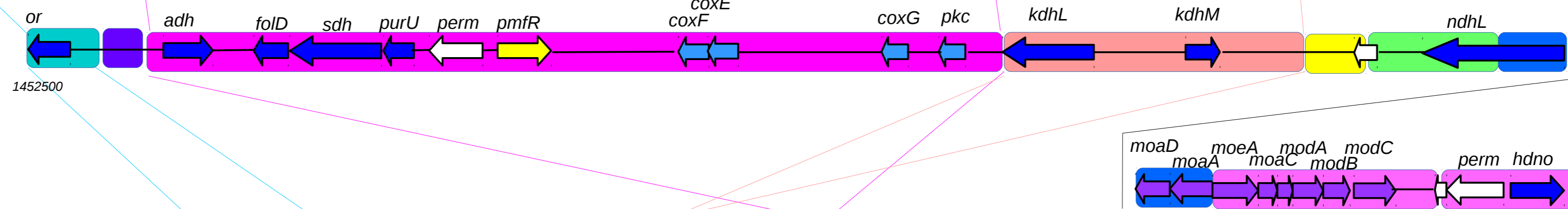
## A. *nicotinovorans* pAO1



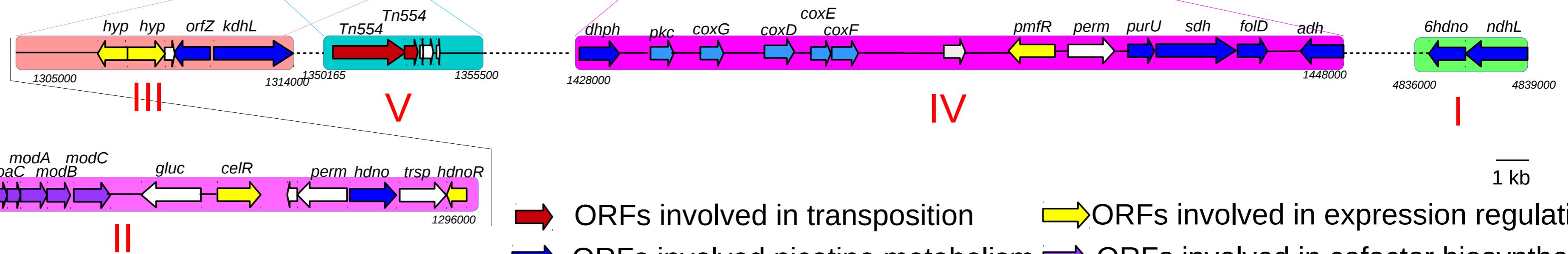
## *Arthrobacter* sp. M2012083



## *Arthrobacter* sp. SJCon

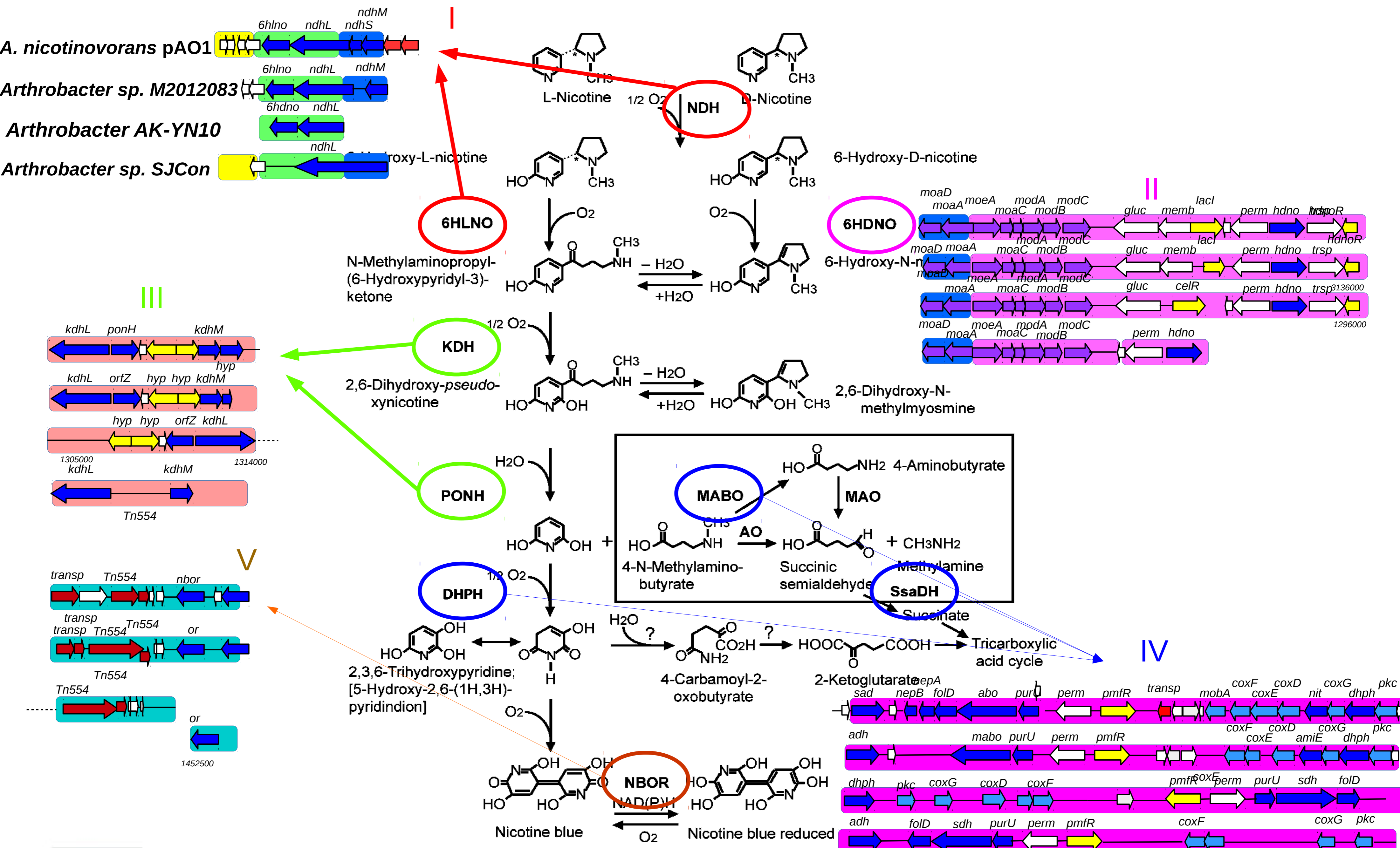


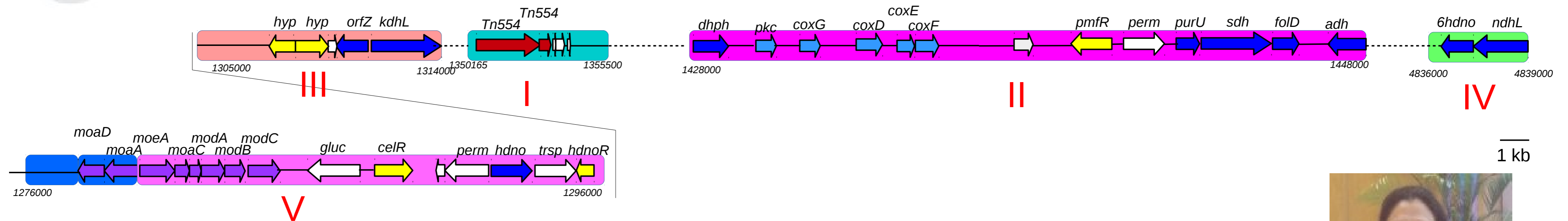
## *Arthrobacter* AK-YN10



- ➔ ORFs involved in transposition
- ➔ ORFs involved in expression regulation
- ➔ ORFs involved nicotine metabolism
- ➔ ORFs involved in cofactor biosynthesis
- ➔ ORFs with unknown function
- Identical colored blocks indicate LCBs

1 kb





**Arthrobacter AK-YN10 strain was a kind gift from Dr. Atya Kapley**

National Environmental Engineering Research Institute, CSIR-NEERI, Nagpur, India



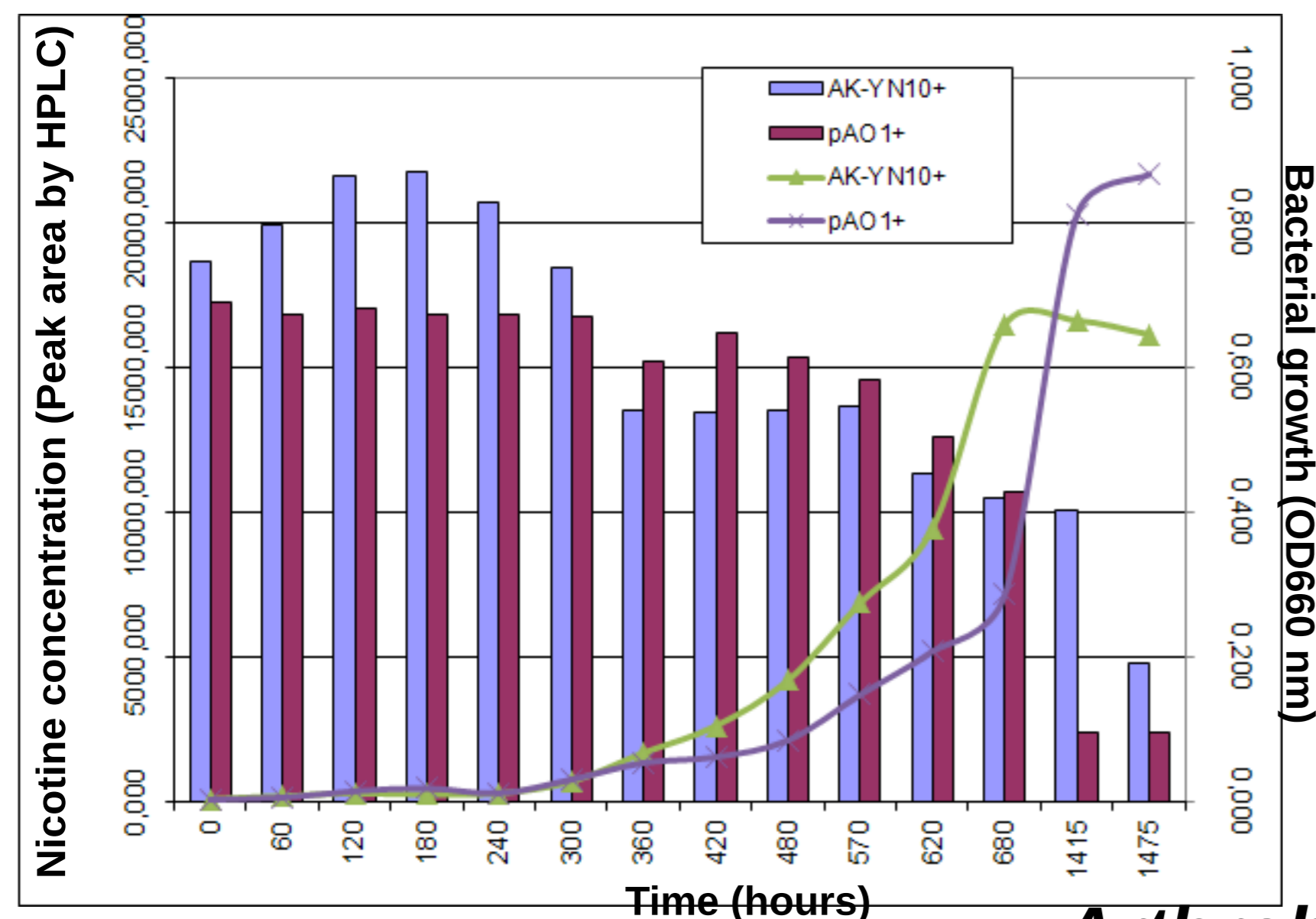
**Nicotine resistance – 6 g/L for AK-YN10 vs: 1,5 g/L for Pseudomonas sp. HF-1**

**3 g/L for Pseudomonas sp. Y22**

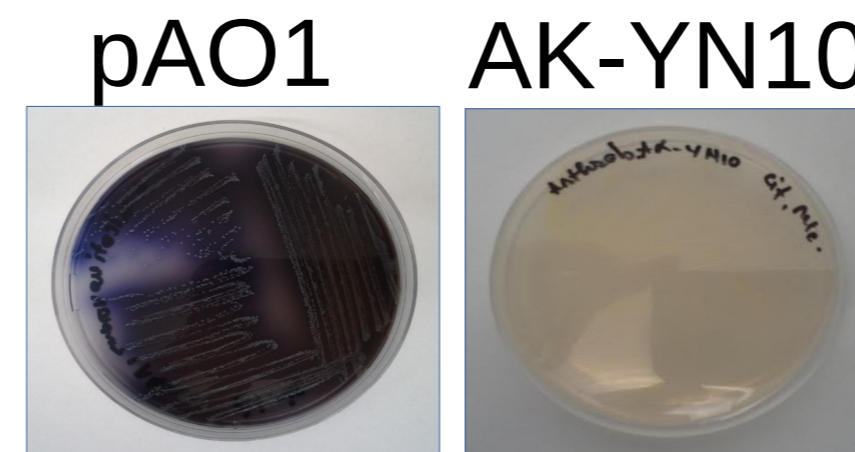
**4 g/L for Pseudomonas geniculata**

**5 g/L for Shinella sp. H2N7**

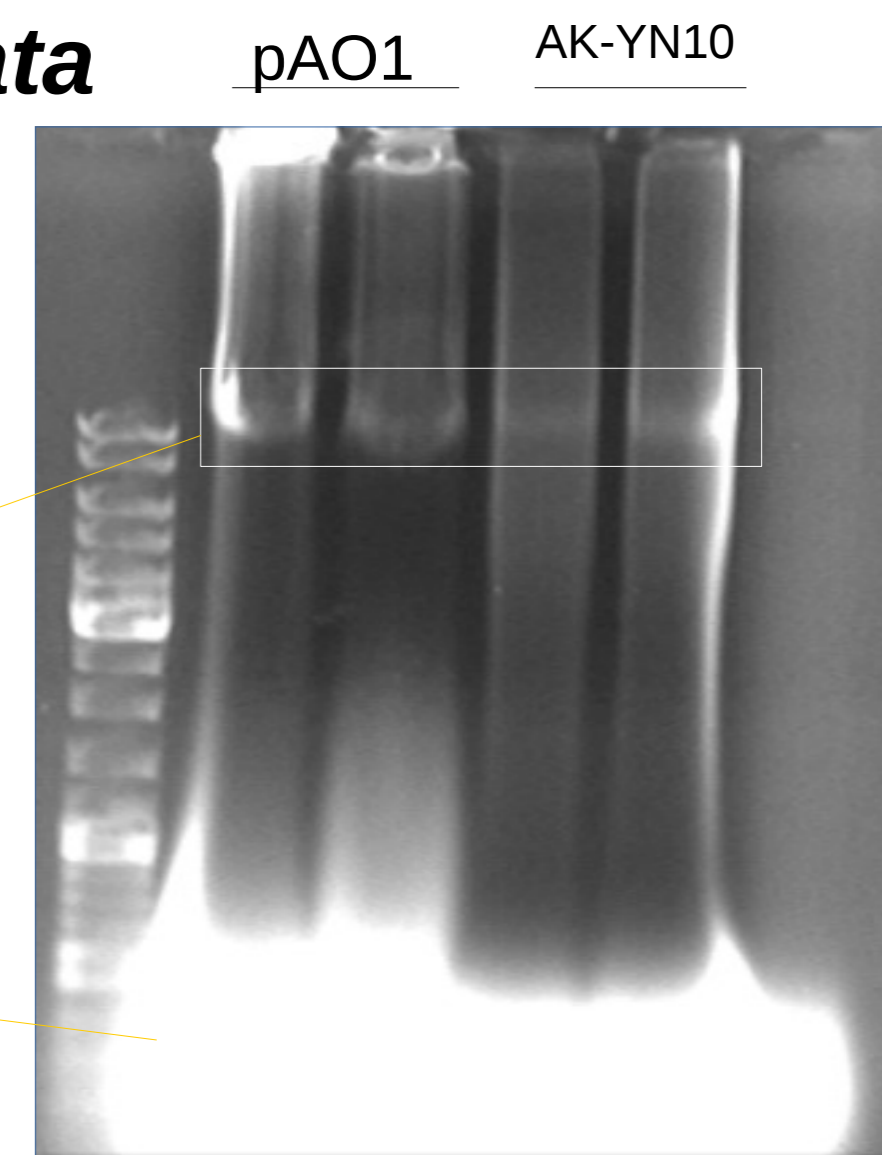
**6 g/L for Arthrobacter nicotinovorans pAO1**



**Arthrobacter AK-YN10 can grow on nicotine containing medium, but does not form the blue pigment.**



Genomic DNA





# Conclusions:



1. Within the *Arthrobacter* genus, the *nic*-gene cluster is not singular to the pAO1 megaplasmid. Three strains (*Arthrobacter sp.* M2012083, *Arthrobacter* AK-YN10, *Arthrobacter sp.* SJCon) have been identified here as containing the *nic*-gene cluster.
2. A modular design for the *nic*-gene cluster can be described, each module coding for a step in the nicotine catabolic pathway
3. *Arthrobacter sp.* AK-YN10 can degrade nicotine, but the catabolic pathway might be slightly different compared to the pAO1 encoded pathway.

## Open questions:

- What is the evolutionary path that led to the *nic*-gene cluster? Did it formed within the *Arthrobacter* genus or was it acquired by horizontal gene transfer? Are the different modules evolving independently?
- What is the origin of the pAO1 megaplasmid as the similarity with other plasmids is low?

## Funny facts:

- genomic data used as input (reference and draft genomes): 60 MB
- number of generated files: approx: 5000
- total amount of generated data (genomes and alignments): 32 GB
- total computation time (assembly and alignments): about 52 hours

## Collaborators:



apl. Prof. Roderich Brandsch, PhD  
Institute of Biochemistry and Molecular Biology,  
Freiburg i. Br., Germany

- nicotine metabolism and pAO1 molecular organization



Assist. Prof. Marius Stefan, PhD  
Biology Faculty, A.I. Cuza University of Iasi

- *Arthrobacter nicotinovorans* manipulations



Prof. Vlad Artenie, PhD  
Biology Faculty, A.I. Cuza University of Iasi

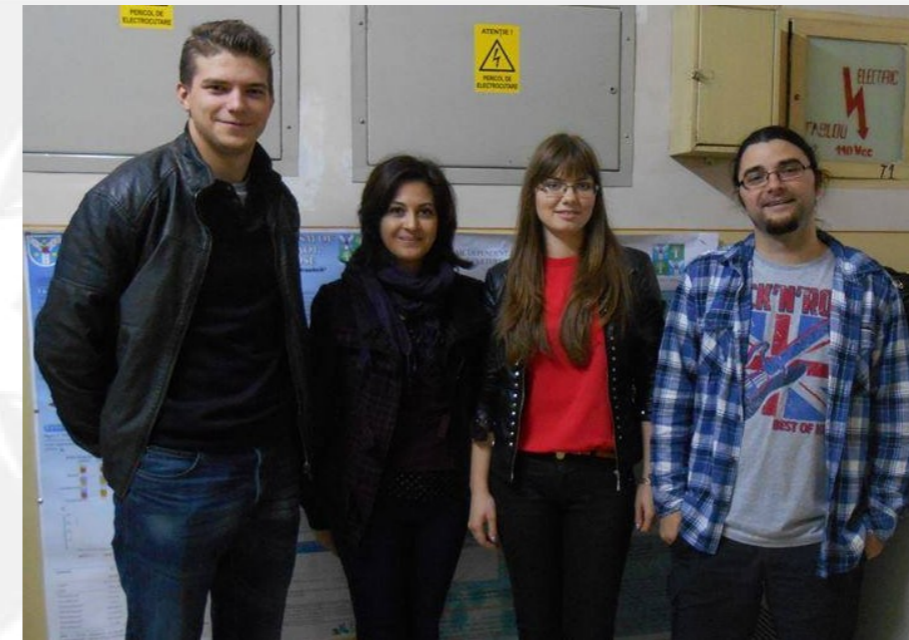
- fruitful talks on enzyme assays and oxidative stress



Prof. Zenovia Olteanu, PhD  
Biology Faculty, A.I. Cuza University of Iasi

- PostDoc coordinator

## Students:



Boiangiu Razvan, B.Sc,  
Iasi, Romania  
Guzun Doina,  
B.Sc, Iasi,  
Romania  
Andreea Andrei, Msc,  
Iasi, Romania



Baumont Victor, Erasmus  
LLP student, IUT Lille,  
France

BIOLOGY FACULTY ALEXANDRU IOAN CUZA UNIVERSITY of IAȘI, ROMANIA

IDENTIFICATION AND CHARACTERIZATION OF BIOLOGICAL ACTIVE MOLECULES

Home People Research Publications Collaborations Media Contact

Welcome

to the website of the "Identification and Characterization of Biological Active Molecules" research group.

About Us

The group is based at the Faculty of Biology, Alexandru Ioan Cuza University of Iasi, Romania. It consists of six academia members and researchers, two technicians as well as students and Ph.D's which share common research interests. The group focuses on biological active molecules with potential applications in biotechnology and this implies the:

- isolation
- identification
- characterization
- study of biological effects in terms of: neurological effects, cytotoxicity, oxidative stress, antimicrobial activity.

News

- 20.03.2014 - Lecture on Nicotine catabolism and its spread by HGT among soil bacteria, Prof. R. Brandsch on 7.04.2014, 10 A.M., B460
- 03.2014 - Web-site launched

Contact

Identification and Characterization of Biological Active Molecules Group

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Funding:



PN-II-RU 337/2010



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GI-2014-02



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