# A highly syntenic *nic*-genes cluster is present in several related bacterial strains

Amada El-Sabeh[1], Andreea-Mihaela Mlesnita[1], Iustin-Tiberius Munteanu[1],
Iasmina Honceriu[1], Fakhri Kallabi[1,2], Razvan-Stefan Boiangiu[1], Marius Mihasan[1]

[1]BioActive research group, Faculty of Biology, Alexandru Ioan Cuza University, Iaşi, Romania;  [2]Laboratory of Human Molecular Genetics,  University of Sfax, Tunisia
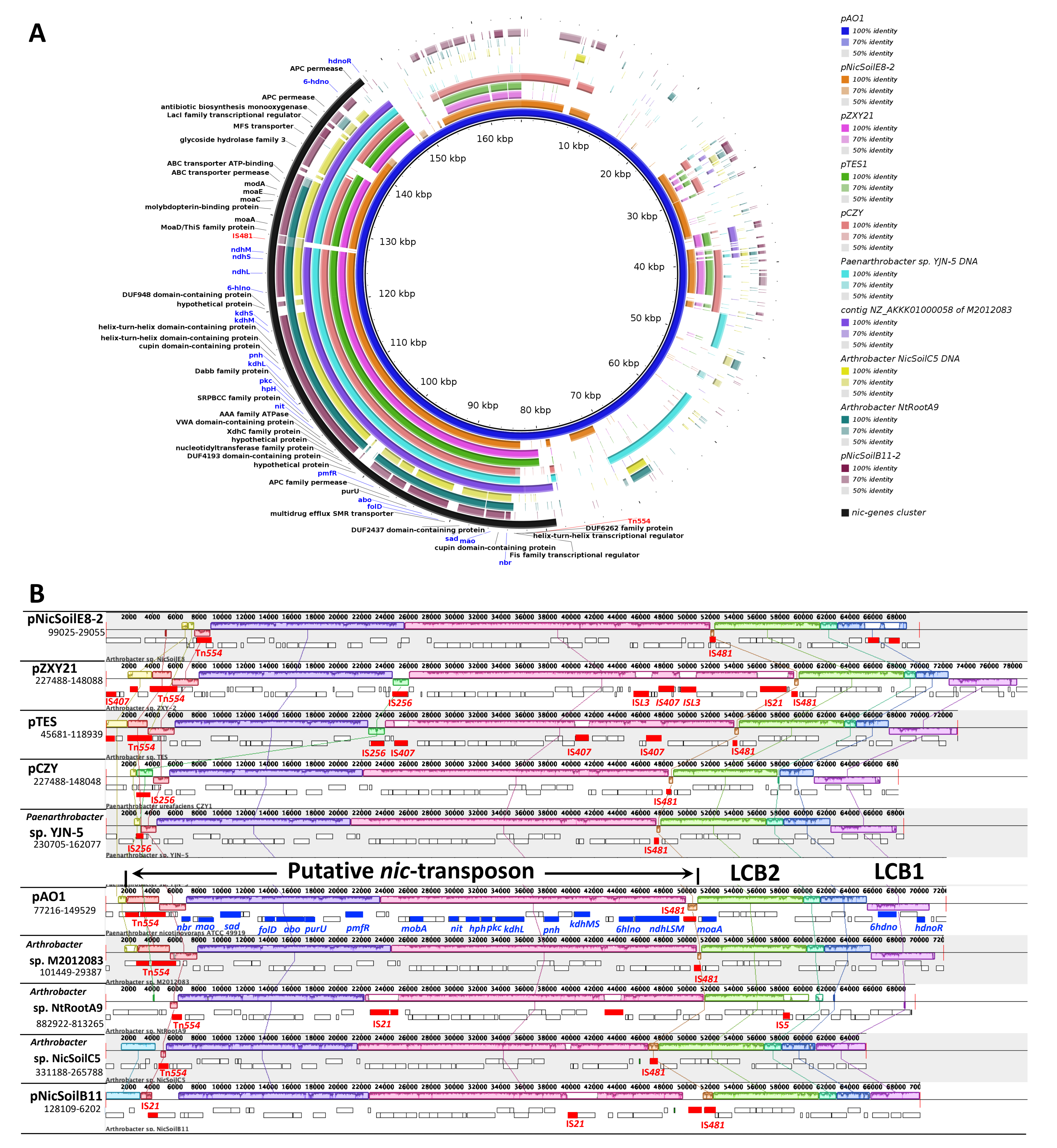
## 1. Introduction

The genes encoding pyridine-pathway for nicotine degradation (*nic*-genes) present in *Paenarthrobacter nicotinovorans* ATCC 49919 can serve as a model for studying the molecular evolution of catabolic pathways.

## 2. Methods

The newly released genome of the strain (GenBank CP089293, CP089294) was used as a reference and an extensive comparative genomics study was performed on a total of 65 related genomes. All pairwise comparisons among the set of genomes were conducted using GBDP available in TYGS, digital DNA-DNA hybridization (dDDH) values and confidence intervals were calculated using GGDC 3.0 and average nucleotide identity (ANI) values were calculated using the OrthoANI algorithm. Species clustering was done using a 70% dDDH cutoff.  Comparative analysis of *nic*-genes cluster was performed with progressive Mauve using the HOXD scoring matrix.

## 3. Results

### Genome wide pairwise comparison



Phylogenetic tree and pairwise comparisons based on both dDDH (d4) and ANI values of 64 genomes of *Paenarthrobacter* strains and of other *Arthrobacter*, *Nocardioides*, and *Rhodococcus* strains which possess the nic-genes.
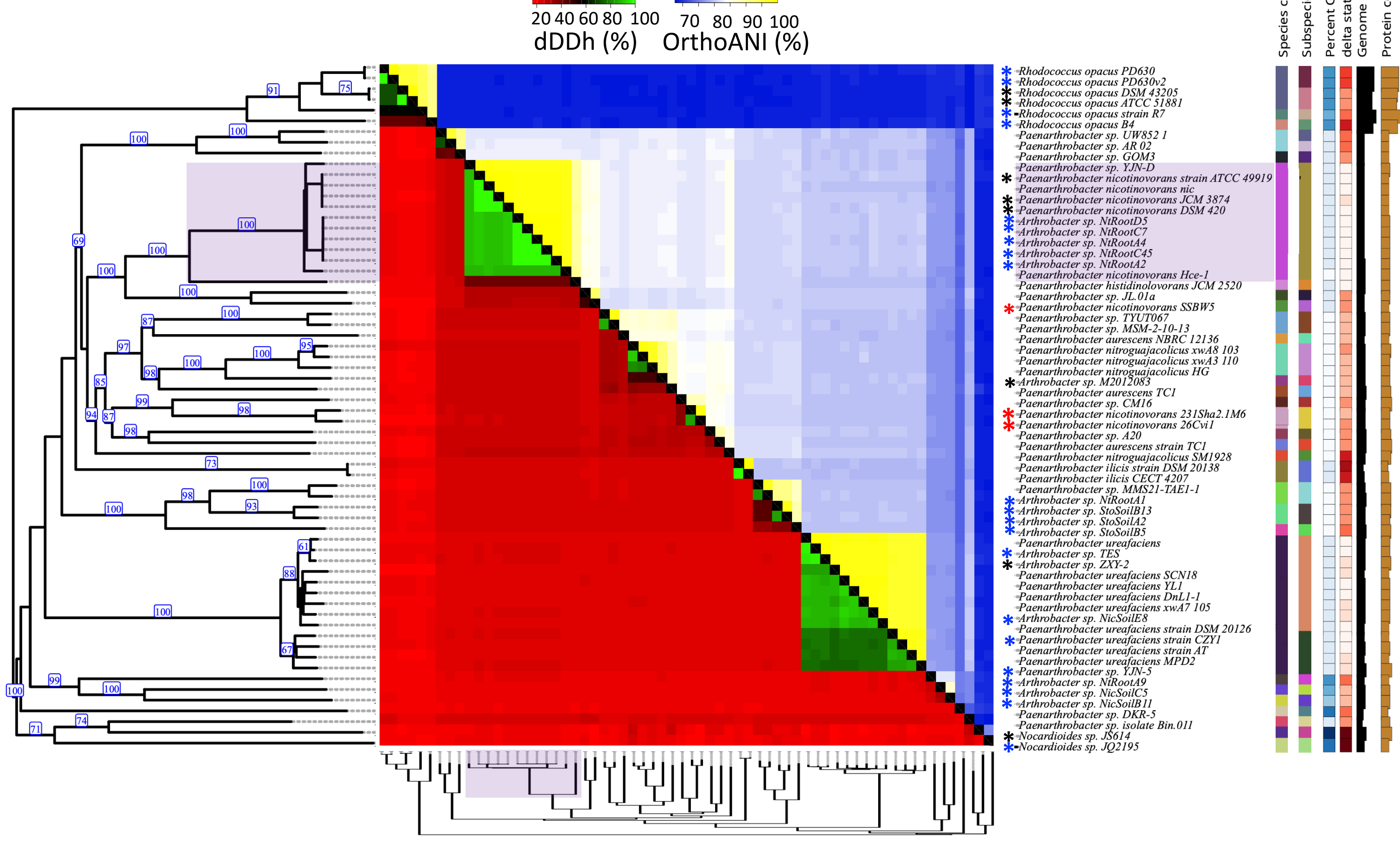
### Highly syntenic *nic*-genes clusters



Nine highly syntenic nic-genes clusters identified in various *Arthrobacter* and *Paenarthrobacter* genomes. (A) BRIG comparative genomic analysis of the pAO1 megaplasmid against the strains harboring the nic-genes listed in the legend. External black circle – the localisation of the nic-genes cluster on pAO1; blue labels – pAO1 genes experimentally related to nicotine metabolism; red labels – genes related to recombination events. (B) Mauve alignments of the nic-genes clusters.
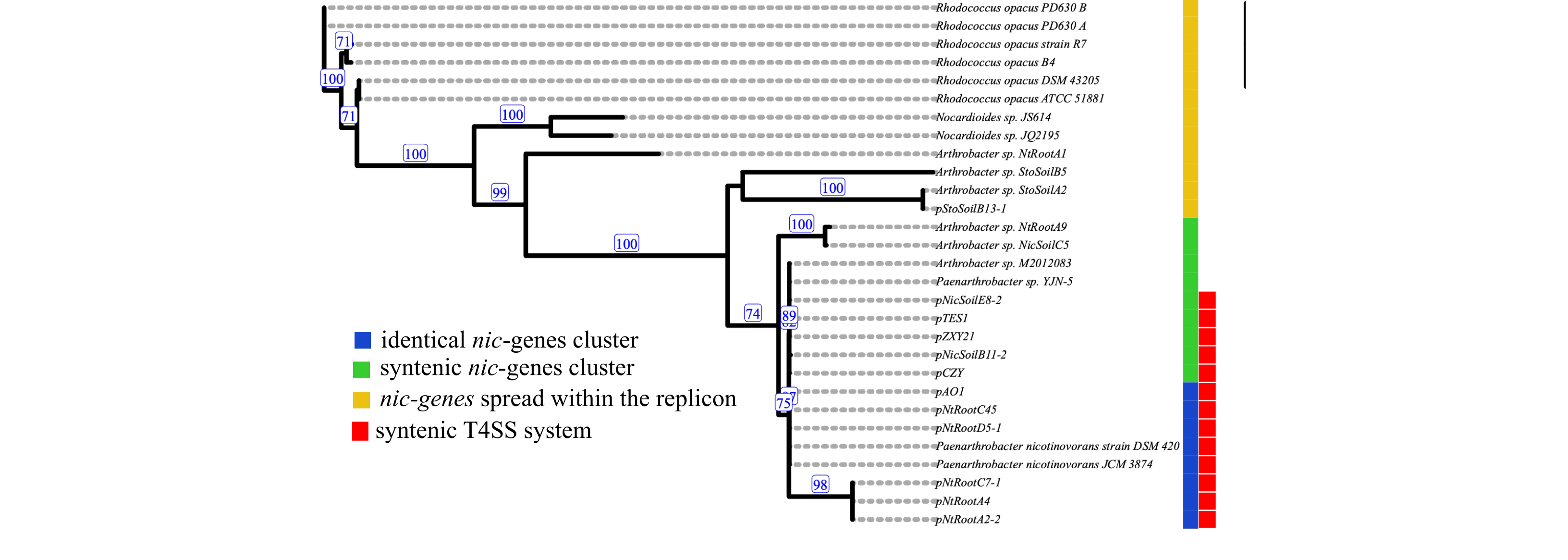


Maximum Likelihood phylogeny of the microbial pyridine pathway for nicotine degradation s reconstructed from the concatenated *purU, pnh, pmfR, ndhL, kdhL* sequences.

### Identical *nic*-genes clusters
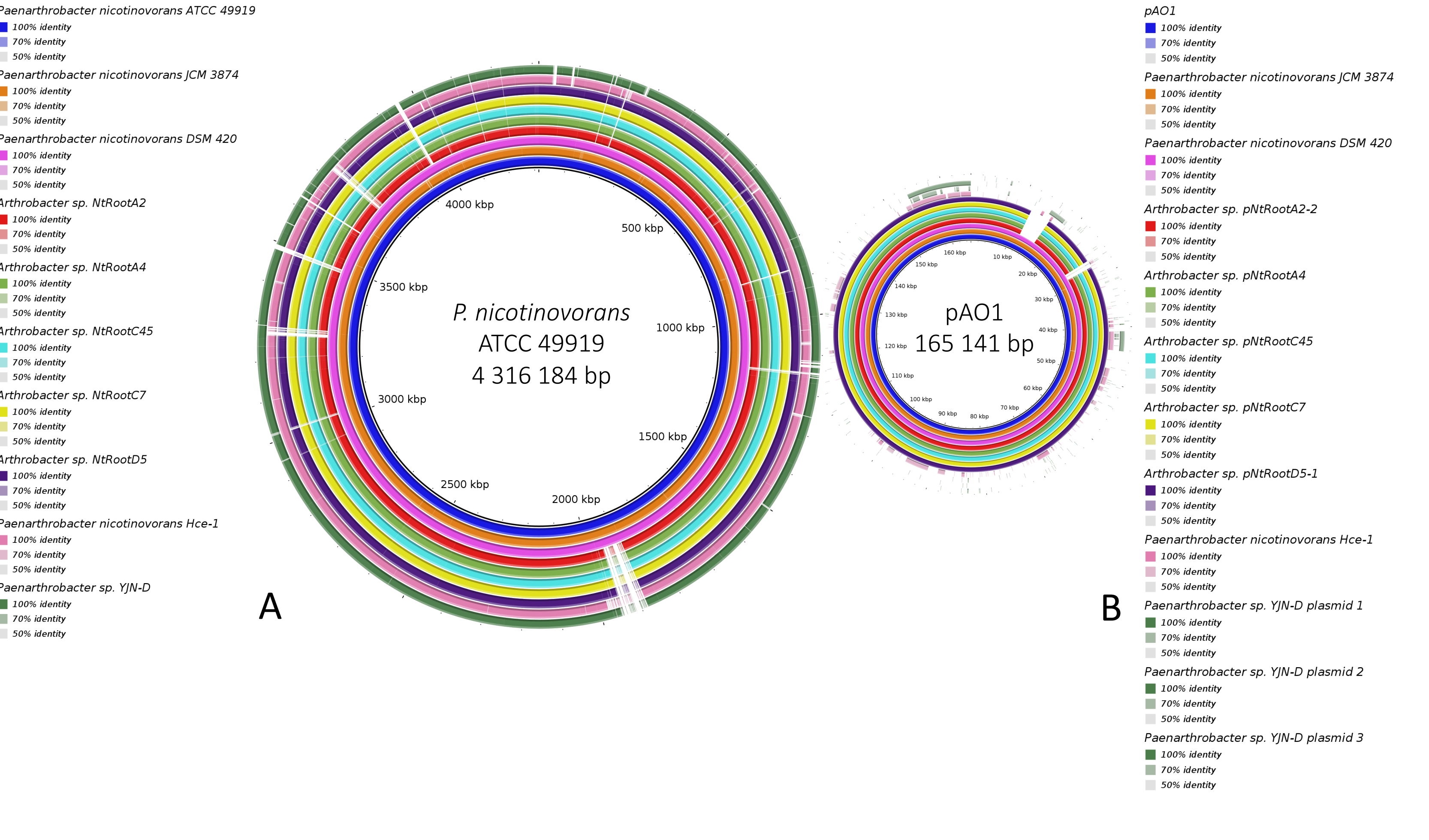


Five *Arthrobacter* strains showing both dDDH and ANI values over the species threshold when compared to *P. nicotinovorans* ATCC 49199 were identified. Five plasmids and two contigs belonging to Arthrobacter and Paenarthrobacter strains were shown to be virtually identical with the pAO1 plasmid of *Paenarthrobacter nicotinovorans* ATCC 49919.

## 4. Conclusions

- Five plasmids and two contigs belonging to *Arthrobacter* and *Paenarthrobacter* strains were shown here to be virtually identical with the pAO1 plasmid of *Paenarthrobacter nicotinovorans* ATCC 49919.
- a highly syntenic *nic*-genes cluster was identified on four plasmids, one contig and four chromosomes
- the *nic*-genes cluster contains two major LCBs that apparently form a putative catabolic transposon

## 5. References

Igloi GL, Brandsch R. Sequence of the 165-kilobase catabolic plasmid pAO1 from Arthrobacter nicotinovorans and identification of a pAO1-dependent nicotine uptake system. J Bacteriol. 2003;185:1976–86.

El-Sabeh A, Honceriu I, Kallabi F, Boiangiu R-S, Mihasan M. Complete Genome Sequences of Two Closely Related Paenarthrobacter nicotinovorans Strains. Microbiol Resour Announc. 2022;11.

Lee I, Kim YO, Park SC, Chun J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol. 2016;66:1100–3

Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M. TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. Nucleic Acids Res. 2022;50:D801–7

Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk v2: memory friendly classification with the genome taxonomy database. Bioinformatics. 2022;38:5315–6