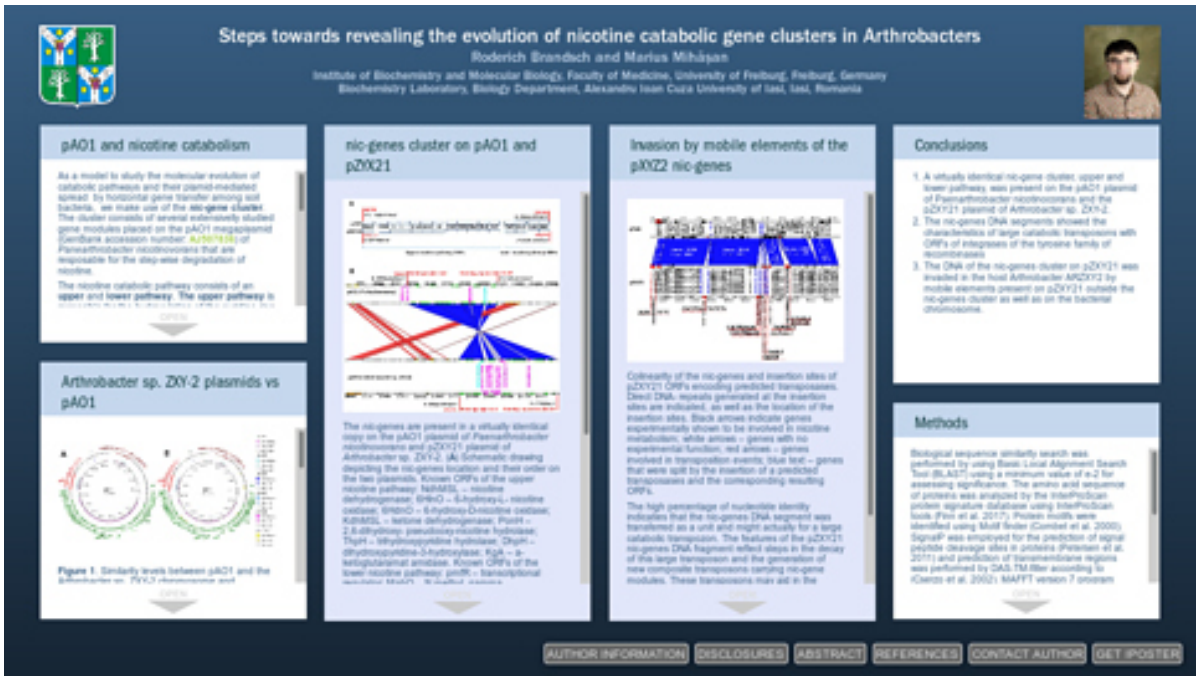
Steps towards revealing the evolution of nicotine catabolic gene clusters in Arthrobacters



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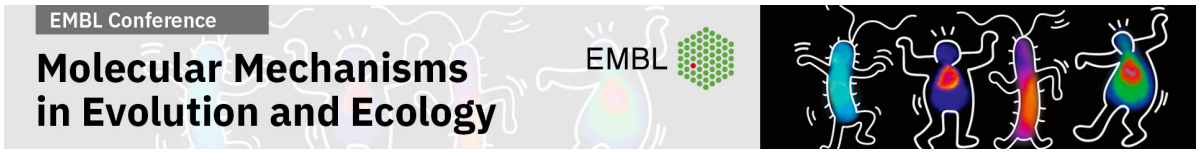


PAO1 AND NICOTINE CATABOLISM

As a model to study the molecular evolution of catabolic pathways and their plamid-mediated spread by horizontal gene transfer among soil bacteria, we make use of the ***nic*-gene cluster**. The cluster consists of several extensivelly studied gene modules placed on the pAO1 megaplasmid (GenBank accession number: **AJ507836 (https://www.ncbi.nlm.nih.gov/nuccore/AJ507836)**) of *Panearthrobacter nicotinovorans* that are resposable for the step-wise degradation of nicotine.

The nicotine catabolic pathway consists of an **upper** and **lower pathway**. **The upper pathway** is resposible for the hydroxylation of the pyridine ring of nicotine, opening of the pyrrolidine ring and cleavage of N-methyl-c-amino-butyrate with the formation of 4,40 ,5,50 -tetrahydroxy-3,30 -di- azadiphenoquinone-(2,20) or nicotine blue (NB) (Brandsch 2006). Recently it was shown that a putative trihydroxypyridine hydrolase (*thpH*) can hydrolytically cleave trihydroxylated pyridines with the formation of 2-oxo-glutaramat (Vaitekunas et al. 2016) which can be deamidated by a *kgA* encoded a a-ketoglutaramat amidase (Cobzaru et al. 2011). Both the *thpH* and *kgA* genes encoded by pAO1 were shown to have a nicotine-dependent expression (Mihasan et al. 2018) and might connect the nicotine pathway to the citric acid cycle.

The lower pathway is resposable for the degradation of N-methyl-c-amino-butyrate with the formation of the end products methylamine and succinic acid that are excreated in the medium or integrated into the general metabolism of the cell respectively.

We have shown that the *nic*-genes spread among soil bacteria by horizontal gene transfer aided by pAO1-related plasmids (Ganas et al. 2008; Cobzaru et al. 2011), with similar *nic*-gens for the upper catabolic pathway beening reported in *Nocardioides* sp. JS614 and *Rhodococcus opacus* B4 (Mihasan and Brandsch 2013). Also, we shown that pAO1 is member of a family of related Arthrobacter catabolic plasmids carrying a predicted type IV secretion system (T4SS) involved in plasmid conjugation and similar replication and partitioning genes (Mihasan and Brandsch 2016).

Here we show that the entire pAO1 nic- genes cluster is present in a identical copy on the pZXY21 plasmid of *Arthrobacter* sp. ZXY-2 (GenBank acces- sion number: NZ_CP017422 (https://www.ncbi.nlm.nih.gov/nuccore/NZ_CP017422)) and that it was invaded by mobile elements. These findings offer a snapshot view on the breakdown of a large catabolic gene cluster into potentially new transposons.

ARTHROBACTER SP. ZXY-2 PLASMIDS VS PAO1

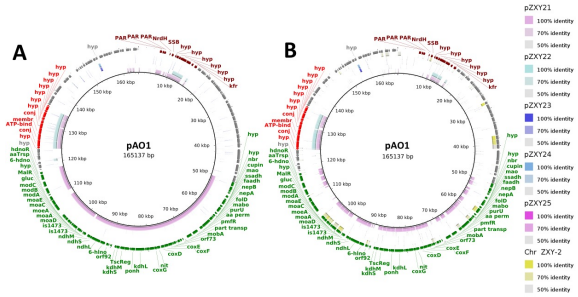


Figure 1. Similarity levels between pAO1 and the Arthrobacter sp. ZXY-2 chromosome and plasmids A) nucleotide and B) protein levels; Reference: pAO1 sequence (GI AJ507836); Querys as concentric circles: pZXY21 (GI:NZ_CP017422), pZXY 22(GI: NZ_CP017423), pZXY23(GI: NZ_CP017424), pZXY24 (GI:NZ_CP017425) pZXY25 (GI:NZ_CP017426) and the Arthrobacter sp. ZXY-2 chromosome (GI: NZ_CP017421); on the external circle relevant pAO1 annotations are depicted; green – nic-genes cluster, red - T4 secretion system, maroon - kfrA related gene cluster involved in plasmid maintenance; genes names correspond to table 1, hyp- hypothetical protein.

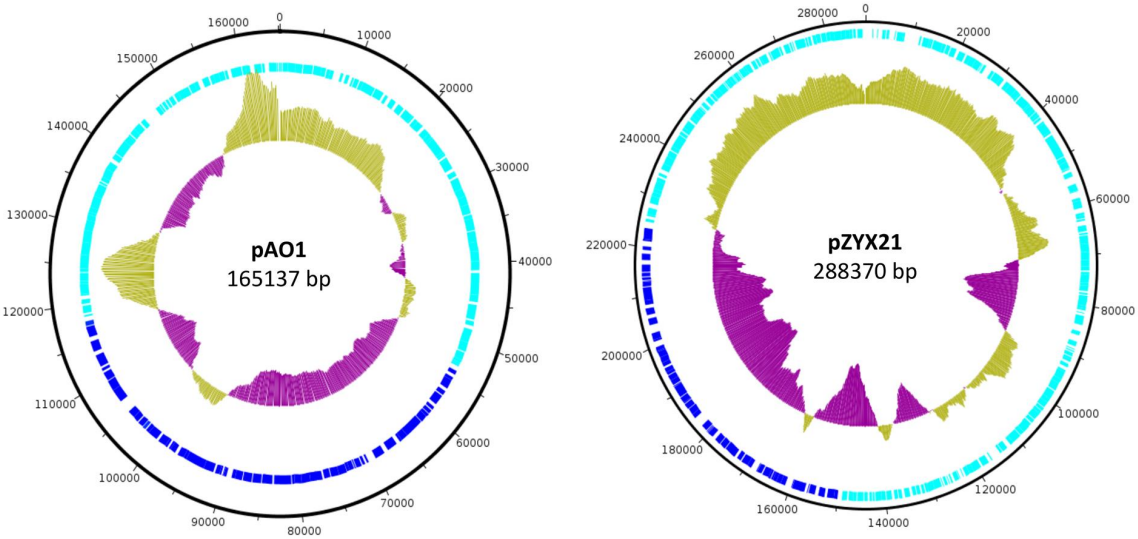
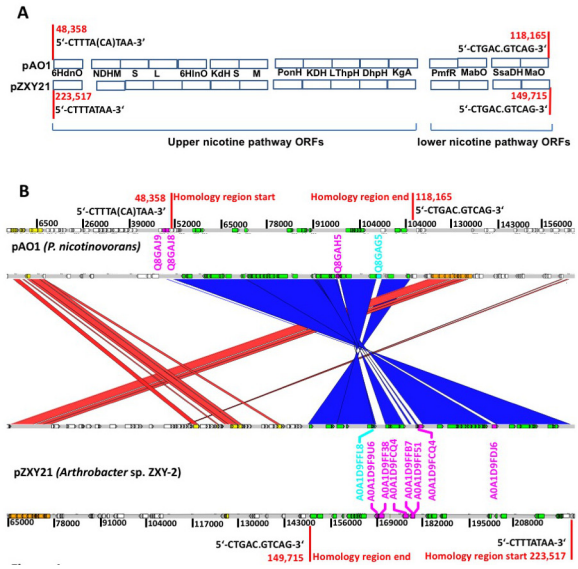


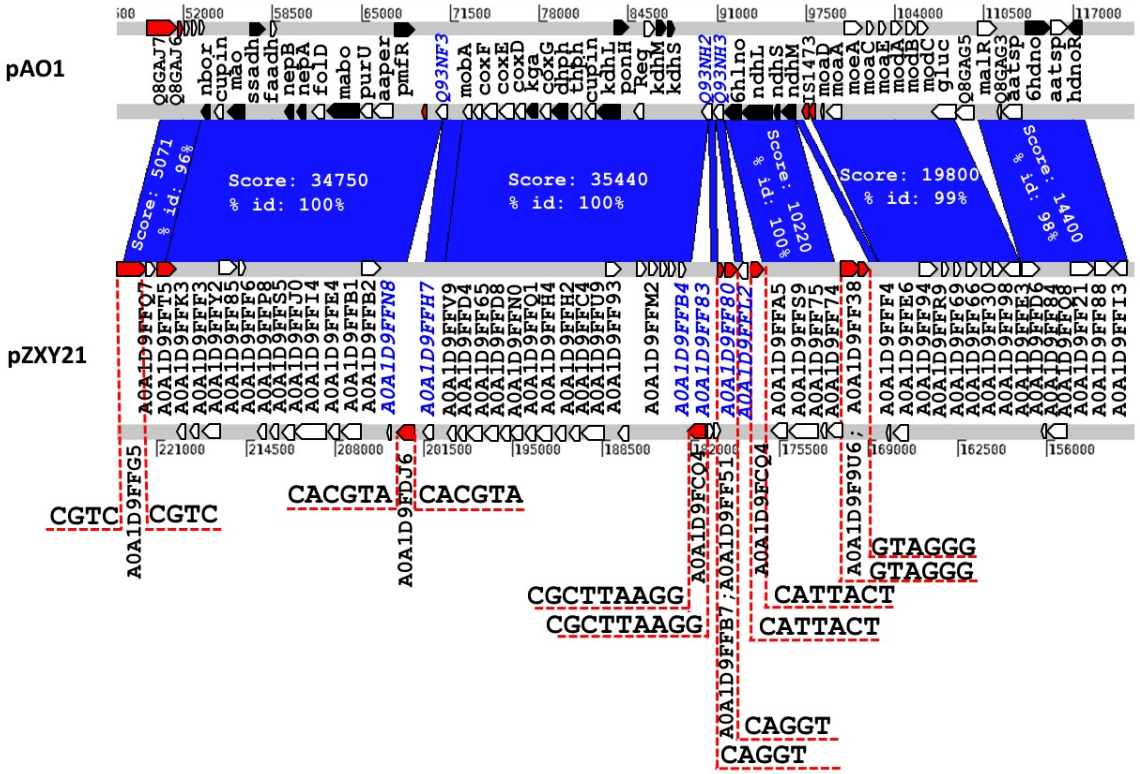
Figure 2. GC content of the pAO1 and pZYX21 plasmids. The inside ring indicates the GC content, magenta - GC content below the plasmid average, yelow - GC content above the plasmid average; middle ring represents ORFs, dark-blue – nic-genes.

NIC-GENES CLUSTER ON PAO1 AND PZYX21



The *nic*-genes are present in a virtually identical copy on the pAO1 plasmid of *Paenarthrobacter nicotinovorans* and pZXY21 plasmid of *Arthrobacter* sp. ZXY-2. (**A**) Schematic drawing depicting the nic-genes location and their order on the two plasmids. Known ORFs of the upper nicotine pathway: NdhMSL – nicotine dehydrogenase; 6HlnO – 6-hydroxy-L- nicotine oxidase; 6HdnO – 6-hydroxy-D-nicotine oxidase; KdhMSL – ketone dehydrogenase; PonH – 2,6-dihydroxy- pseudooxy-nicotine hydrolase; ThpH – trihydroxypyridine hydrolase; DhpH – dihydroxypyridine-3-hydroxylase; KgA – a- ketoglutaramat amidase. Known ORFs of the lower nicotine pathway: pmfR – transcriptional regulator; MabO – N-methyl- gamma-aminobutyrtae oxidase; SsaDH – succinate semialdehyde dehydrogenase; MaO – gamma-aminobutyrate oxidase; (**B**) Gene co-linearity and nucleotide sequence identity between pAO1 and pZXY21; green colored arrows – nic-gene cluster, orange colored arrows – type IV secretion system, yellow colored arrows – kfrA related gene cluster involved in plasmid maintenance; UniProtKB IDs are indicated for ORFs that are not found on both plasmids, magenta text and arrow – transposases, cyan text and arrows – unknown function (A0A1D9FFL8, predicted transposase related to Q8GAH5/IS1473; Q8GAG5 predicted major facilitator superfamily permease). Nucleic acid similarities (BLASTN comparisons showing at least 95% identity) are depicted as solid lines between sequences, where red indicates regions of identity in the same orientation and blue indicates regions of identity with the opposite orientation.

INVASION BY MOBILE ELEMENTS OF THE PXYZ2 NIC-GENES



Colinearity of the nic-genes and insertion sites of pZXY21 ORFs encoding predicted transposases. Direct DNA- repeats generated at the insertion sites are indicated, as well as the location of the insertion sites. Black arrows indicate genes experimentally shown to be involved in nicotine metabolism; white arrows – genes with no experimental function; red arrows – genes involved in transposition events; blue text – genes that were split by the insertion of a predicted transposases and the corresponding resulting ORFs.

The high percentage of nucleotide identity indicaties that the *nic*-genes DNA segment was transferred as a unit and might actually for a large catabolic transpozon. The features of the pZXY21 *nic*-genes DNA fragment reflect steps in the decay of this large transposon and the generation of new composite transposons carrying nic-gene modules. These transposons may aid in the transfer of individual parts of the nic-genes DNA to other replicons (related Arthrobacter plasmids, chromosomes or plasmids of other soil bacteria) by horizontal gene transfer such as it happened in *Rhodococcus opacus* B4 and *Nocardioides* sp. JS614.

CONCLUSIONS

1. A virtually identical *nic*-gene cluster, upper and lower pathway, was present on the pAO1 plasmid of *Paenarthrobacter nicotinovorans* and the pZXY21 plasmid of *Arthrobacter* sp. ZXY-2.
2. The nic-genes DNA segments showed the characteristics of large catabolic transposons with ORFs of integrases of the tyrosine family of recombinases;
3. The DNA of the nic-genes cluster on pZXY21 was invaded in the host Arthrobacter ARZXY2 by mobile elements present on pZXY21 outside the nic-genes cluster as well as on the bacterial chromosome.

METHODS

Biological sequence similarity search was performed by using Basic Local Alignment Search Tool (BLAST) using a minimum value of e-2 for assessing significance. The amino acid sequence of proteins was analyzed by the InterProScan protein signature database using InterProScan tools (Finn et al. 2017). Protein motifs were identified using Motif finder (Combet et al. 2000). SignalP was employed for the prediction of signal peptide cleavage sites in proteins (Petersen et al. 2011) and prediction of transmembrane regions was performed by DAS-TM-filter according to (Cserzo et al. 2002). MAFFT version 7 program (Yamada et al. 2016) and Cobalt (Papadopoulos and Agarwala 2007) were used for multiple alignments of amino acids and nucleotides. Gene synteny was analyzed and visualized with ACT (Carver et al. 2005) and progressive Mauve (Darling et al. 2010). GC content analysis were performed and visualized with Artemis (Carver et al. 2012). BLAST results were also visualized with BRIG (Alikhan, 2011).

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Mihasan, M.; Babii, C.; Aslebagh, R.; Channaveerappa, D.; Dupree, E. & Darie, C. C., Proteomics based analysis of the nicotine catabolism in P. nicotinovorans pAO1, Scientific Reports, 2018, 8, Article number: 16239
Hritcu, Lucian, Radu Ionita, Diana Elena Motei, Cornelia Babii, Marius Stefan & Marius Mihasan. 2017. “Nicotine versus 6-Hydroxy-l-Nicotine against Chlorisondamine Induced Memory Impairment and Oxidative Stress in the Rat Hippocampus.” Biomedicine & Pharmacotherapy 86: 102–8.
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ABSTRACT

The genes encoding the nicotine catabolic pathway from the pAO1 megaplasmid of Paenarthrobacter nicotinovorans are organized into several gene nicmodules responsible for the stepwise degradation of L and Dnicotine to nicotine blue. Nicmodules with variable degrees of identity and synteny has been shown to exist in different Grampositive soil bacteria, making the pAO1 a good model for studying the evolution of catabolic pathways. Here we show that a virtually identical copy of the pAO1 nic-modules is also present on the on pZXY21 plasmid of Arthrobacter sp. ZXY2. The identical stretch of DNA is 70 kb long and contains 50 nicotine degradation related genes with a nucleotide sequence identity of over 99%. Outside this stretch of DNA, the sequence identity of the two plasmids drops significantly. At the 5´ end of identical DNA stretch are located the ORFs of two predicted integrases, suggesting that the entire nicgenes DNA fragment is a large catabolic transposon. The nicgenes on pZXY21 are interspersed by mobile elements encoding transposases of various IS families. Insertion of these IS elements disrupts nicotine degradation and divide the nicgenes DNA into potentially new transposons. This finding may illustrate how nicotine catabolic genes can be mobilized and spread by horizontal gene transfer to other soil bacteria.

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