

Comparative analysis of the *nic*-gene cluster within the *Arthrobacter* genus Andreea Andrei, Marius Mihăşan



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## Introduction

The pAO1 megaplasmid of Arthrobacter nicotinovoras harbors two different catabolic pathways: one for degradation of xylose (xyl operon) and one for nicotine degradation (nic-gene cluster). Our previous work has shown that the megaplasmid is responsible for spreading the nicotine degrading ability to various Gram positive soil bacteria, such as Rhodococus or Nocardioides. The recent advances in NextGen sequencing has led to the deposition of 37 genomes and 24 plasmids belonging to the Arthrobacter genus. Interestingly, the pAO1 shows low levels of sequence similarity with the other Arthrobacter plasmids, but shares most of its nic-genes with three Arthrobacter genomes: Arthrobacter sp. M2012083 (GI:NZ AKKK00000000), Arthrobacter sp. SJCon (GI: NZ AOFD0000000) and Arthrobacter sp. AK-YN10 (GI: NZ AVPD00000000). The current study attempts to make an evolutionary analysis of the nic-cluster tacking into account only the gene arrangement and collinearity of the open reading frames.

# Methods

The three draft genomes of the above mentioned Arthrobacter strains were assembled based on five reference genomes (Arthrobacter aurescens TC1 (GI:NC 008711), Arthrobacter chlorophenolicus A6 (GI:NC 011886), Arthrobacter arilaitensis Re117 (GI:NC 014550), (GI:NC 015145), Arthrobacter phenanthrenivorans Sphe3 Arthrobacter nitroguajacolicus Rue61a (GI:NC 018531) by using the AlignContig module from MAUVE v.2.1.3. For each draft genome, the sequence of aligned contigs was further uploaded to the RAST server for automated annotation. The final genomes were further aligned using MAUVE, BLAST and BRIG. The Arthrobacter sp. AK-YN10 strain was a kind gift from Dr. Atya Kapley (National Environmental Engineering Research Institute, CSIR-NEERI, Nagpur, India) and was grown on citrate medium supplemented or not with nicotine. Nicotine resistance was assayed using the broth microdilution method. The nicotine concentration in the medium was falowed by HPLC of a Bischoff dual-pump system equipped with a DAD detector and a Nucleodur RP C18ec column. An isocratic elution was employed using  $1 \text{mN H}_2\text{SO}_4$  at a flow rate of 1 ml/min.

#### Metabolic profiles of the annotated Arthrobacter genomes



#### **Nic-gene cluster consists of five modules**



### Arthrobacter AK-YN10 can degrade nicotine



#### Conclusions

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. A modular design for the *nic*-gene cluster can be described, each module coding for a step in the nicotine catabolic pathway. *Arthrobacter* AK-YN10 can degrade nicotine, but the catabolic pathway might be slightly different compared to the  $pAO^{1}$  encoded pathway.

#### References

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