Nicotine-Induced Proteome of *Arthrobacter nicotinovorans* pAO1

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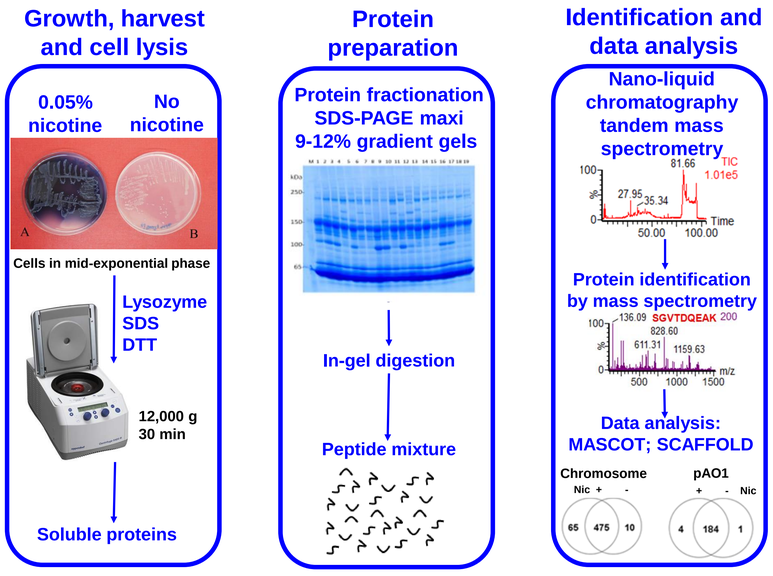
Abstract

The current work attempts to identify all the nicotine induced proteins from *Arthrobacter nicotinovorans* pAO1+ using SDS-PAGE, in gel digestion, and nanoliquid chromatography-tandem mass spectrometry (nano-LC-MS/MS). The extracts from the bacteria grown on nicotine-containing media showed several extra bands in the range of 60 and 30 kDa. One of these nicotine-related bands was identified as a KatA, a chromosomal heme-binding catalase. Full-scale proteomic analysis is currently under way. Identification of all the key regulators and enzymes of the nicotine catabolism in *Arthrobacter nicotinovorans* would allow a better understand smoking addiction, but to also might offer a way to manipulate the nicotine catabolic pathway to treat tobacco waste and convert it into green chemicals.

Introduction

Large quantities of waste with high concentrations of nicotine are generated during the tobacco-manufacturing process and are simply discarded into the environment. The Gram-positive soil bacterium *Arthrobacter nicotinovorans* is able to degrade nicotine using a catabolic pathway encoded by the pAO1 megaplasmid (Figure 1). The purpose of this study is to identify all the regulators and enzymes related the nicotine catabolism in *A. nicotinovorans*. This would provide a way to manipulate the nicotine catabolic pathway to treat tobacco waste and convert it into green chemicals.

Methods



Results

SDS-PAGE of proteins from the bacteria grown on nicotine-containing media showed several extra bands in the range of 60 and 30 kDa. One particularity of *A. nicotinovorans* proteome is the high abundance of small molecular weight proteins of around 14 kDa (Fig 1).

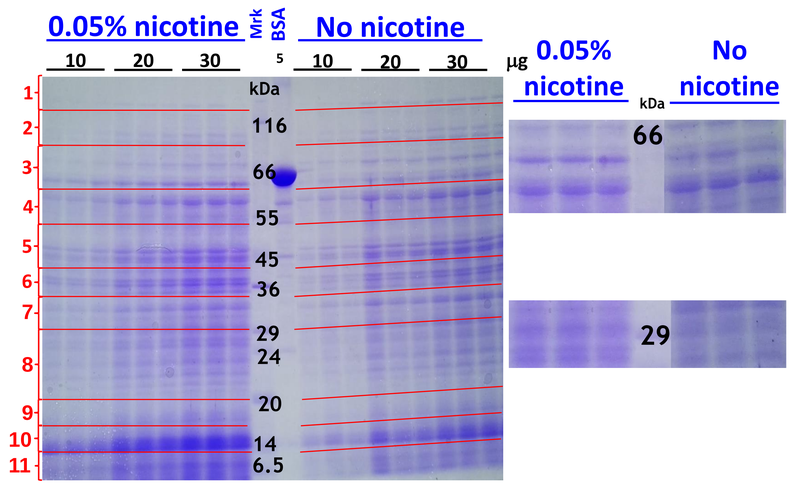


Figure 1. SDS-PAGE of *Arthrobacter nicotinovorans* proteins grown on citrate medium supplemented with 0.05% nicotine (left) or citrate medium without nicotine (right). Details of the 66-55 kDa and 24-29 kDa areas of the same gel showing different band patterns.

The high intensity, low MW band (gel piece 10 in Figure 1) contains the same *Arthrobacter* related proteins in both the nicotine-induced and not-induced samples as depicted in table 1:

Table 1. Hits in both nicotine induced and not-induced *Arthrobacter* samples

Protein	GI	Function
50S ribosomal protein L7/L12	gi|489900399	induces the catalytically active conformation of EF-Tu and EF-G
50S ribosomal protein L32	gi|489902608	-
Nitroreductase	gi|495536387	reduction of nitro-compounds using NAD(P)H in a obligatory two-electron transfer
Integration host factor	gi|496741372	DNA replication, site-specific recombination, and gene expression

Nano-LC-MS/MS analysis of the 60 kDa MW band (gel piece 3 in Fig 1) indicated that the chromosomal heme-binding catalase KatA (gi|500012190) is expressed only in the nicotine-induced bacteria (Fig 2). The KatA might help protecting the bacterial cells against the reactive oxygen species that were shown to be generated during the one-electron reductions of nicotine end-products.

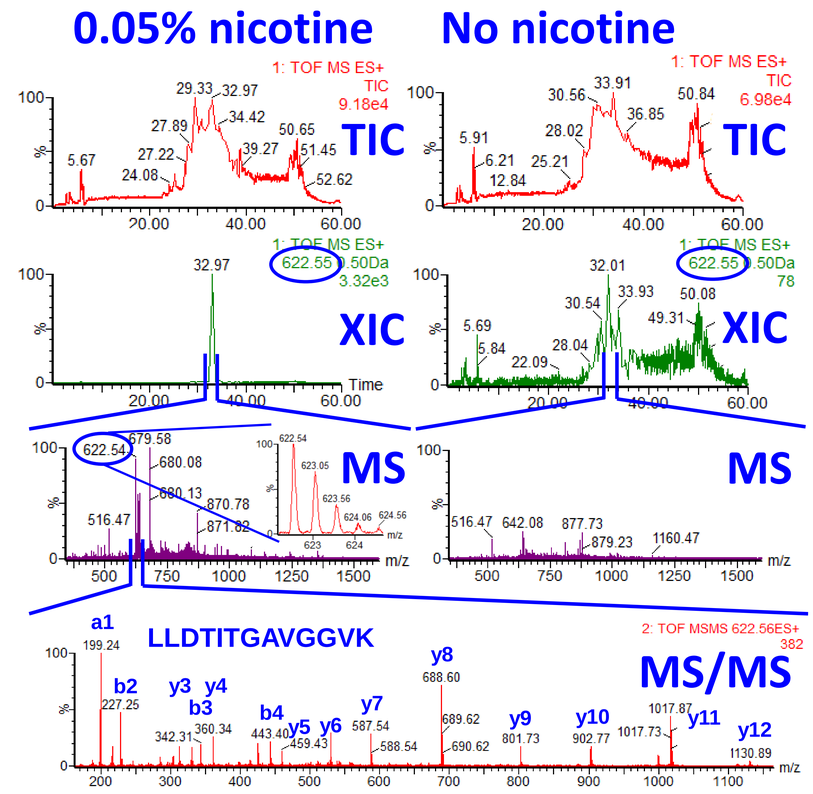


Figure 2. TIC, XIC, MS and MS/MS of a m/z 622.54 peptide related to the catalase enzyme.

Conclusions

- We have successfully identified some differences between the nicotine-induced and not-induced proteome of *A. nicotinovorans*
- The differences are not always related to the pAO1 megaplasmid indicating a tight integration of the nicotine pathway
- This is an ongoing investigation

References

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