

Proteomic Analysis of Nicotine Metabolism in *Paenarthrobacter Nicotinovorans*

Paenarthrobacter nicotinovorans is a soil Gram-positive Nicotine-Degrading Microorganism (NDM) that has applications in the eco-friendly conversion of nicotine-containing waste into green-chemicals. The nicotine catabolic genes have been sequenced, but little is known on how the cells cope with the accumulation and toxicity of the resulting by-products. Shotgun proteomics was used to identify all the proteins expressed by *P. nicotinovorans* in the presence of nicotine and perform a time-based analysis. Cells were grown on citrate, 0.005% nicotine, and a combination of the two. Cells were harvested and lysed at 3 different time intervals: 7, 10, and 24 hours post-inoculation. Cell-free extracts were separated on 9–16% SDS-PAGE maxi gels. After reduction, alkylation and in-gel digestion using trypsin, the resulting peptide mixture was analyzed on a NanoAcquity UPLC (Waters, USA) coupled to a Q-TOF Xevo G2 MS (Waters). Data analysis was performed using Mascot v.2.5.1 (Matrix Science, UK) and Scaffold (Portland, USA). This approach allowed us to identify a total of 915 proteins grouped into 584 non-redundant clusters with an FDR of 0.3%. The differences in protein abundance showed that deamination is preferred in the nicotine pathway when citrate is present. Several putative genes have been shown to have a nicotine-dependent expression, including a putative enzyme that might cleave the pyridine ring with the formation of alpha-keto- glutaramate. Moreover, the strong up-regulation of malate dehydrogenase and a D-3-phosphoglycerate dehydrogenase correlate well with the production of alpha-keto-glutamarate. The proteomics data has the PRIDE dataset identifier PXD012577.

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