Experimental evidence of a xylose-catabolic pathway on the pAO1 megaplasmid of Arthrobacter nicotinovorans

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Abstract

The soil bacterium Arthrobacter nicotinovorans carries the pAO1 catabolic megaplasmid which enables it to grow on xylose (Ighis and Brondich, 2003). Besides the well-characterized pathway for nicotine degradation (Brondich et al., 2006), pAO1 carries a gene cluster of a hypothetical pathway for carbohydrate utilization (figure 1). This cluster consists of ORFs of a transcriptional regulator, of a sugar ABC-transporter and of several putative dehydrogenases and oxidoreductases. Previously, we established that the pAO1 orfD gene encodes an aldohex-4-dehydrogenase (Mihăsăń et al., 2008) and orfR encodes an sugar dehydrogenase. The current work is focused on experimental identification of the catabolic pathway substrate of this latter pathway.

**Methods**

Directional gene cloning was performed using standard methods using the pGEX3 plasmid, protein expression was achieved using auto-inducible medium as described elsewhere (Mihăsăń, Ungurâns & Artenie, 2007). Protein purification was done using standard IMAC techniques (Assieul M Frederick et al., 2002) on Fast-Flow Ni-chelating Sepharose (Amersham Biosciences, Sweden). Molecular weight determination of the native protein was performed by GPC, using a prepacked HiLoad 16/60 Superdex 200 column calibrated with GPC Wide Range calibration kit (Amersham, Biosciences, Sweden). Antibodies against purified proteins were developed in rats and used for Western-Blots. Carbohydrate metabolism assay was performed with the API 50CHL (Biomerieux, France) per producer’s indications.

**Results**

GntR was purified to homogeneity as a 29 kDa His-tagged recombinant protein. As indicated by GPC it consists of a monomeric protein with a native molecular weight of 32 kDa. The specific UV/Vis spectra showed only a single peak at 280 nm common for all proteins and did not indicated the presence of any colored cofactors. This is in good agreement with the fact that Pdcβ-family proteins contain a winged helix-turn-helix (wHTH) domain responsible for DNA binding, and not a Zn-finger or any other metal containing domains.

OxRE was purified as a 45 kDa His-tagged protein was purified. The native molecular mass of 163 kDa determined by GPC indicated that the protein was a tetramer in solution. Metal content analysis of the purified preparations (table 1) showed that the enzyme binds 2 Zn2+ atoms/protein monomer.

**Conclusions**

Although our previous in-silico blind docking experiments indicated tagatose as the putative ligand for several proteins in the pathway (Mihăsăń, 2010), the current work showed that tagatose is degraded by both the pAO1+ and pAO1- strains. Nevertheless, the docking scores always placed xylose among the top five ligands. Here, the Western-Blots show a clear connection between the pAO1 encoded proteins and the D-xylose metabolism and thus identifying the substrate of the second catabolic pathway coded by the A. nicotinovorans megaplasmid.

**Bibliography**


Acknowledgements. This work was supported by CNCSIS-UEFISCUS, project number PN II- RU 337/2010.