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

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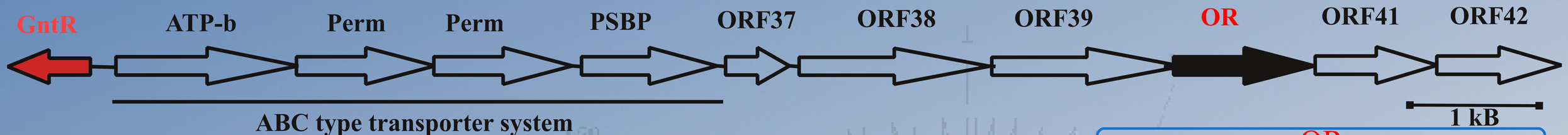
Introduction

The soil bacterium Arthrobacter nicotinovorans carries the pAO1 catabolic megaplasmid which enables it to grow on nicotine (Igloi and Brandsch, 2003). Besides the well-characterized pathway for nicotine degradation (Brandsch, 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 orf39 gene encodes an aldehyde-dehydrogenase (Mihasan et al., 2008) and orf40 encodes an sugar dehydrogenase. The current work is focused on experimental identification of the catabolic pathway substrate of this latter pathway.

Methods

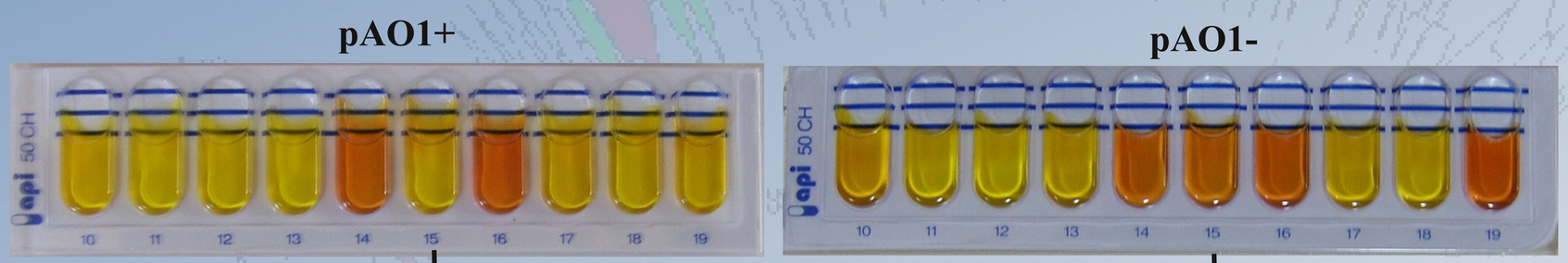
Directional gene cloning was performed using standard methods *using the pG6EX3 plasmid, protein expresion* was achived using auto-inducible medium as described elsewhere (Mihasan, Ungureanu & Artenie, 2007). *Protein purification* was done using standard IMAC techniques (Ausubel M Frederick et al., 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 calobration 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.



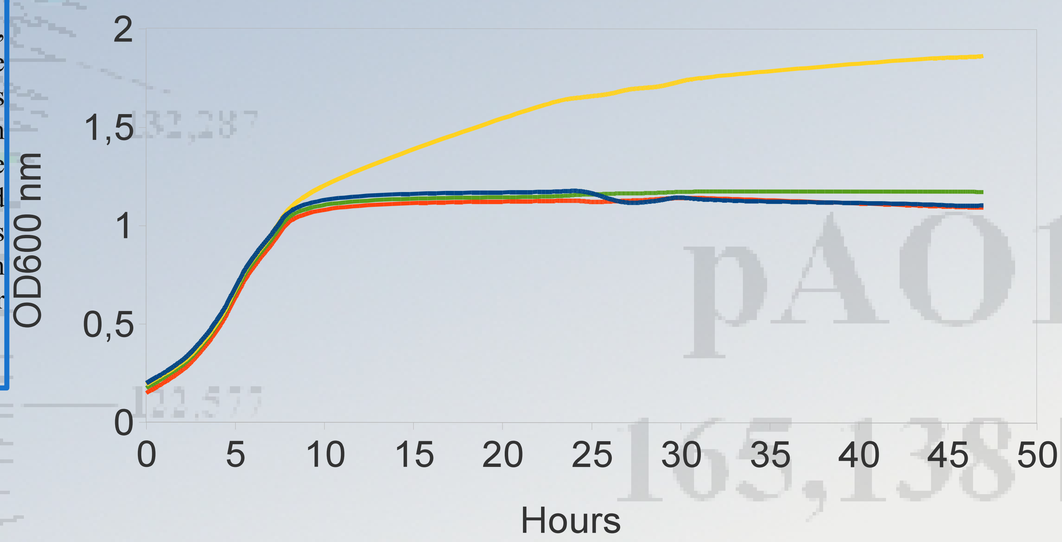
GntR
- transcriptional factor

OR
- oxidoreductase
- contains the consensus sequence 119-AGKHIFTEKP-128 similar to the sugar-dehidrogenases motif - AGKHVxCEKP

Arthrobacter nicotinovorans



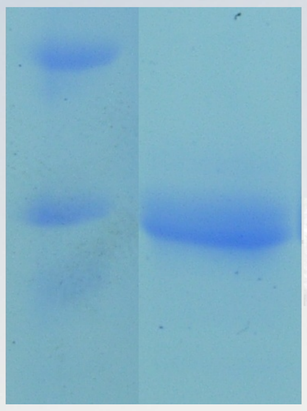
GntR was purified to homogenity as a 29 kDa His-tagged recombinant protein. As indicated by GPC, it consists of a monomeric protein with a native molecular weight of 32 kDa. The specific UV/Vis spectra showed only a single peak at 280 nm common for all proteins and did not indicated the presence of any colored cofactors. This is in good agreement with the fact that PdhR-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.

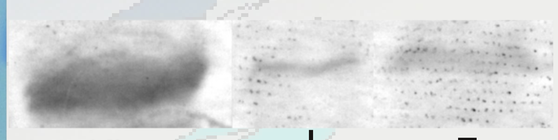


***A. nicotinovorans* pAO1+ and pAO1- growth curve on citrate medium and on citrate medium + D-Xylose**

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 preparates (table 1) showed that the enzyme binds 2 Zn2+ atoms/protein monomer.

IMAC purified GntR

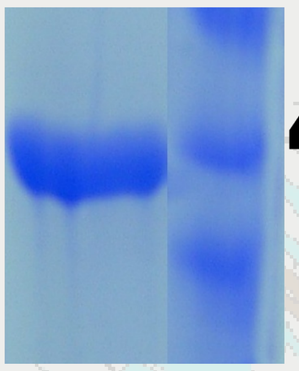




CFE extracts of *Arthrobacter nicotinovorans* pAO1 growth on citrate media (-) and on citrate media supplemented with D-xylose (+)

CFE extracts of *Arthrobacter nicotinovorans* pAO1 growth on citrate media (-) and on citrate media supplemented with D-xylose (+)

IMAC purified OxRe



Conclusions

Although our previous in-silico blind docking experiments indicated tagatose as the putative ligand for several proteins in the pathway (Mihasan, 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

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3. Pettersen E. F., Goddard T. D., Huang C. C., Couch G. S., Greenblatt D. M., Meng E. C. & Ferrin T.E. 2004. UCSF Chimera--a visualization system for exploratory research and analysis. J. Comput. Chem. 25:1605-12.

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