CLONING AND PURIFICATION OF A PUTATIVE REPRESSOR PROTEIN FROM *ARTHROBACTER NICOTINOVORANS* PAO1

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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. 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, 2010) and *orf40* encodes an sugar dehydrogenase. Here we focus on further characterization the ORF32 protein and elucidation of its possible role in the cell. By cloning the gene in the plasmid vector pH6EX3, we were able to express it as a recombinant His-tagged protein and to easily purify it to homogenity.

Table 1. Oligo-nucleotides used for the isolation and cloning of *orf32*

Name	Sequence
ForwGntRBam	5-ggccgaggatccatggacg-3
RevGntRXho	5-cgctaccactcgaggctgacc-3

Methods

Isloation and cloning of orf32. The orf32 was isolated by PCR using the primers in table 1 and a suspension of *Arthrobacter nicotinovorans* cells as template. Directional gene cloning using the degenerated primers from table 1. Directional cloning (Sambrook J, Fritsch EF, Maniatis T,1989) of the fragment containing the orf32 in the pH6EX3 vector was achieved by using BamHI şi XhoI (NEB, U.K) enzymes and Rapid DNA ligation Kit, Roche). Transformed *E. coli* XL1 Blue competent cells were selected on plates containing ampiciline (50 microg/ml) and the recombinant plasmid was checked for the presence of insert by restriction enzyme digestion.

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).

Gel permeation chromatography (GPC) was performed on a 16/20 Sephadex 200 pg (Amersham Biosciences) column attached to Pharmacia LKB FPLC system. The column was first calibrated using bluedextran, aldolase (158 kDa), ovalbumine (47 kDa), and chimiotripsinogen (25 kDa). Protein concentration was assayed using the dye-binding method of Bradford (Bradford,1976). SDS-PAGE was performed using the discontinuous system of Laemlli fallowing the procedure described by Sambrook, 1989.

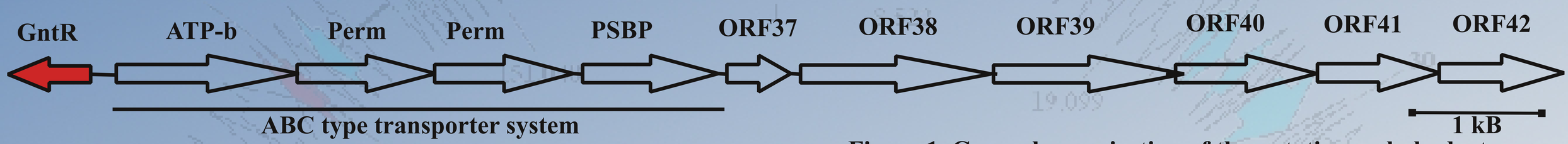
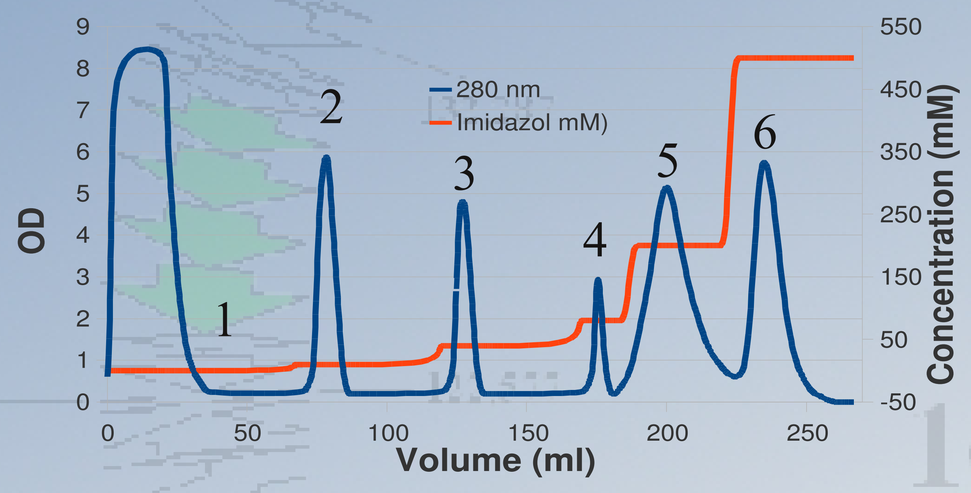


Figure 1. General organisation of the putative carbohydrate utilisation gene cluster from pAO1

Purification and native MW determination



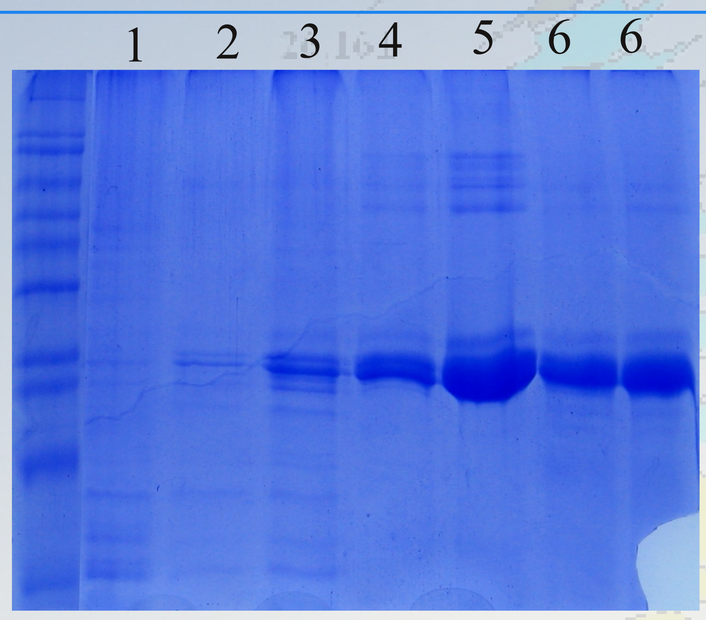


Figure 2. Orf32 encoded protein was purified close to homogenity. Left- IMAC chromatography Right- SDS-PAGE with the purified protein

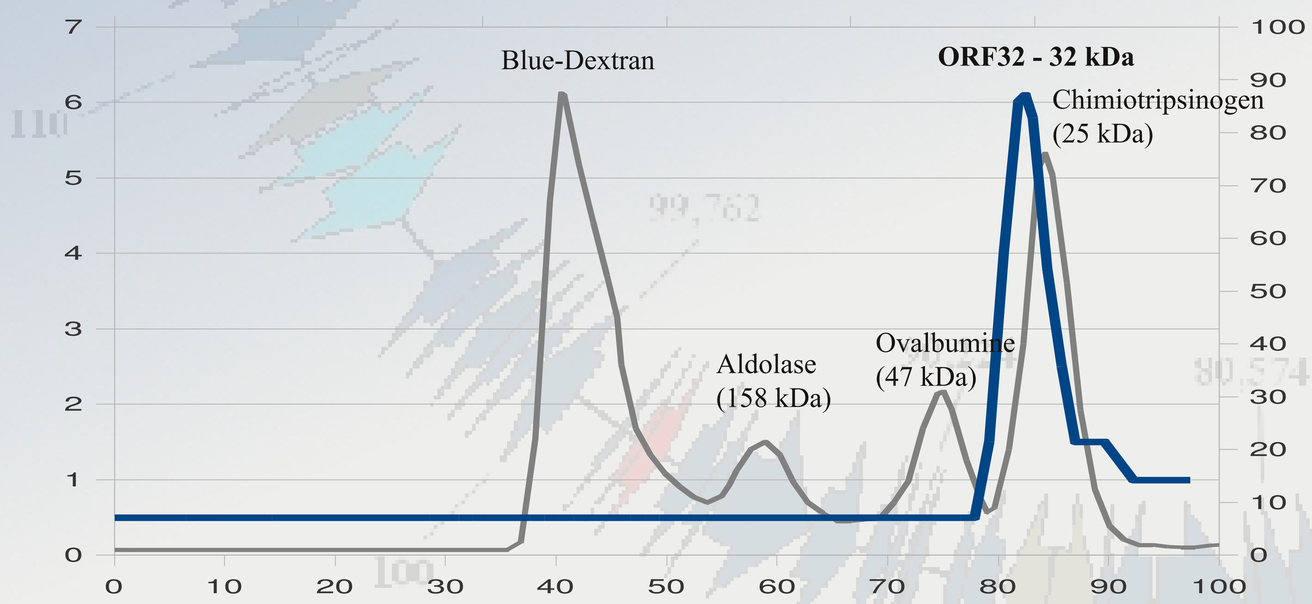


Figure 3. Determination of native molecular mass of ORF32 protein.

1.6 mg purified ORF40 protein was injected on a HiLoad 16/60 Superdex 200 previosly calibrated using Bluedextran, Aldolase (158 kDa), Ovalbumine (47 kDa) and Chimiotripsinogen (25 kDa).

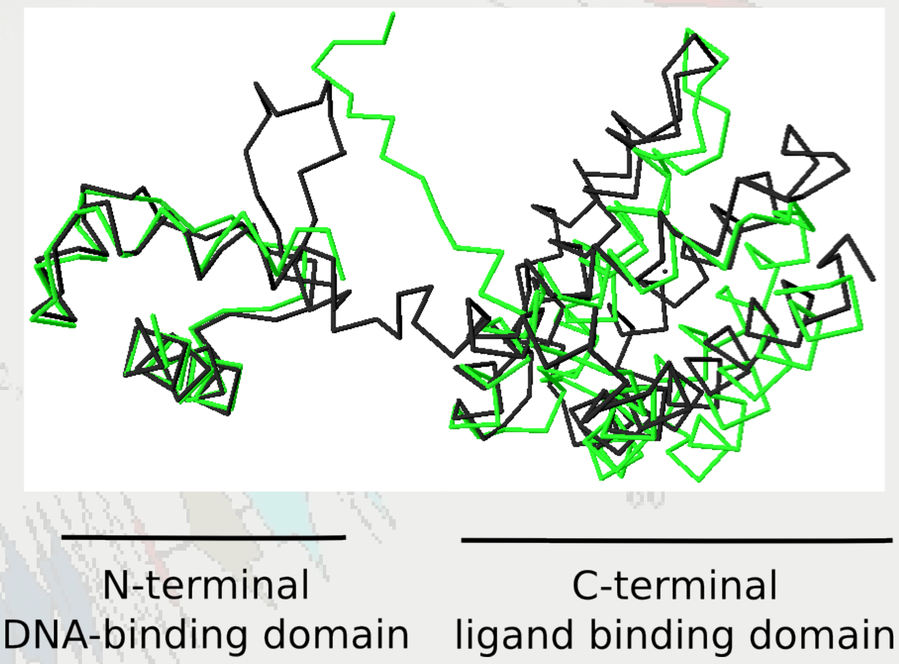


Figure 4. Superimposition of the computer-generated pAO putative transcriptional factor GntR (green) and *Corynebacterium glutamicum* LdlR transcriptional factor (black; PDB code: 2di3).

Conclusions

The orf32 gene of Arthrobacter nicotinovorans pAO1 encodes a monomeric 32 kDa protein containing no metal ions. Due to its position and orientation, it is most probable the repressor protein for the whole putative carbohydrate catabolic operon.

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