**Introduction**

The soil bacterium *Arthrobacter nicotinovorans* carries the pAO1 catabolic megaplasmid which enables it to grow on nicotine (Iqbal and Brandisch, 2003). Besides the well-characterized pathway for nicotine degradation (Brandisch, 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 ofT39 gene encodes an aldehyde-dehydrogenase (Mihășan, 2010) and ofT40 encodes a sugar dehydrogenase. Here we focus on further characterization of the ORF32 protein and elucidation of its possible role in the cell. By cloning the gene in the plasmid vector pH6E3X, we were able to express it as a recombinant His-tagged protein and to easily purify it to homogeneity.

**Methods**

**Isolation 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 pH6E3X vector was achieved by using BamHI yi XhoI (NEB, U.K.) enzymes and Rapid DNA ligation Kit, Roche. Transformed E. coli XL1 Blue competent cells were selected on plates containing ampicilin (50 microg/mL) and the recombinant plasmid was checked for the presence of insert by restriction enzyme digestion.

**Protein expression** was achieved using auto-inducible medium as described elsewhere. (Mihășan, Ungureanu & Artenie, 2007) Protein purification was done using standard IMAC techniques (Auseubel M Fredericke 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 blue dextran, aldolase (158 kDa), ovalbumine (47 kDa), and chymotripsinogen (25 kDa). Protein concentration was assayed using the dye-binding method of Bradford (Bradford,1976).

**SDS-PAGE** was performed using the discontinuous system of Laemmli following the procedure described by Sambrook, 1989.

**Purification and native MW determination**

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

**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.

**Bibliography**


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