CLONING AND EXPRESSION OF A β-MANNOSIDASE- ENCODING GENE FROM *Cellulomonas uda* in *E. coli*

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Introduction. Manans are one of the major hemicellulose constituent. They are composed of homogeneous backbone of β-1,4-linked mannose residues with ramifications of galactose or glucose. The complete conversion of requires the activity of endomannanases(EC: 2.2.1.78), β-manosidases (EC: 3.2.1.25) and α-galactosidase (EC. 3.2.1.22) (1). β-manosidase is widely used in biopulping and biofuels. It is also attractive its use in transglycosilation for obtaining oligossacharides. *Cellulomonas uda* has a cellulolytic and hemicellulolytic system hemicelulases however; there is not studies of the β-manosidase in this microorganism (2).
The aim of this work is to clone and express the β-manosidase encoding gene of *C. uda* in *E. coli*.

Methods. Sequence was obtained by direct PCR and Genome Walking (Clontech). Two Genome Walking libraries were used. ORF encoding the mannosidase from *C. uda*, was subcloned in pQE30 (Qiagen) resulting in the *pQE30_CumanA*. *E. coli* strain M15 was transformed with *pQE30_CumanA*. Enzymatic activity of β-manosidase was determined with *p*-nitrophenyl-β-mannopyranoside.

Results. *CumanA* gene from *C. uda* was isolated and sequenced. Sequence obtained shown a gene of 2574 nucleotides, codifying a protein of 858 amino acids. Identity analysis of *Cu*ManA with other mannosidases is shown **Fig. 1**

Microorganism	Identity %	SCORE	E-VALUE
Cellulomonas fimi	**64**	**1066**	**0.0**
Beutenbergia cavernae	**62**	**994**	**0.0**
Streptomyces sp.	**58**	**957**	**0.0**
Nocardiopsis dassovillei	**56**	**933**	**0.0**

Fig.1 Identity of β-manosidasa from*Cellulomonas uda.* After Clustal W alignment.

Amino acid sequence analysis of of CuManA shown three conserved domains: galactose binding domain, catalytic domain IgG like domain and transglucosidase domain **Fig. 2**

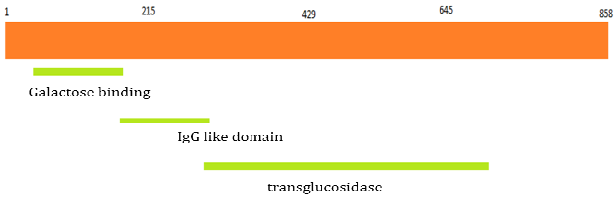

Fig.1 Conserved domains in β-manosidase from *Cellulomonas uda.* Galactose binding domain (41- 316),catalitic domain IgG like (209-316), transglucosidase domain (324-662).

CumanA was cloned in expression vector pQE30_Xa. Restriction analysis is shown in **Fig. 3.** The expected fragment was obtained after digestion (2.8kb)

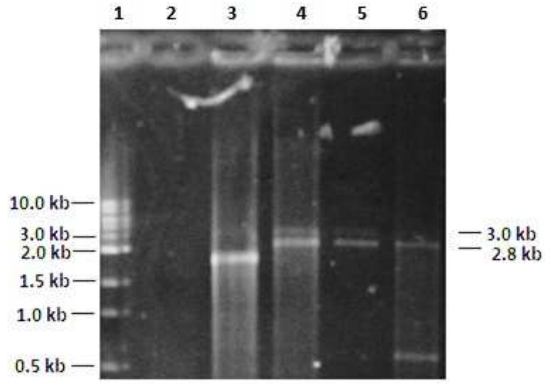

Fig. 3 Restriction analysis of *pQE30_CumanA*.
Lane1: 1 kb ADN ladder; lane 3-6. Restriction analyisis *HindII* and *StuI*.

Solubility assays were done, the recombinant protein was present in inclusion bodies. After solubilization volumetric activity was detected to be 123.87 U/ml.

Conclusions. The *CumanA* gene from *C. uda* encods a protein of 858 aminoacids with 64% identity to mannosidase from *C.fimi*. The *CumanA* gene was expressed in *E. coli* and the recombinant protein presented mannosidase activity of 123.87 U/ml.

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