



## A novel $\beta$ -galactosidase from *Arthrobacter sulfonivorans*: purification, characterization and gene cloning

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### Abstract

Process schemes yielding  $\beta$ -galactosidase of different purity grade were designed, the enzyme properties were determined. It was the first isolation and investigation of the gene encoding *Arthrobacter sulfonivorans* extracellular  $\beta$ -galactosidase.

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Published online: 27 January 2017

doi:10.24190/ISSN2564-615X/2017/01.19

### Introduction

$\beta$ -galactosidase (EC 3.2.1.23) catalyzes not only hydrolysis of terminal non-reducing ( $\beta$ -D-galactose residues in ( $\beta$ -D-galactopyranosides, including the disaccharide lactose (1,4-O- $\beta$ -D-galactopyranosyl-D-glucose), but also transgalactosylation reaction that produces galactooligosaccharides of different polymerization degree. Therefore in biotechnology beta-galactosidases are mainly employed for hydrolysis of lactose to D-glucose and D-galactose in various milk products, making it available to a large number of adults and children that are lactose intolerant. The enzyme is also used for prevention of lactose crystallization in frozen and condensed milk products, for the reduction of water pollution caused by whey and for production of galactooligosaccharides recognized as a growth-stimulating factor for intestinal bifidobacteria and promoting human and animal health (1-3). It was found recently that beta-galactosidase could be used for lactulose production *in vitro* (4).

Although  $\beta$ -galactosidases from prokaryotes have been well-studied, very few data have been reported on its enzyme and in *Arthrobacter* genus in particular.

*Arthrobacter sulfonivorans*  $\beta$ -galactosidase of unique for prokaryotes extracellular localization may find application in the food industry for manufacturing lactose-free dairy products and in pharmacology as a bioactive principle of medicines prescribed for patients suffering from lactase deficiency. The study was aimed at cloning of the gene encoding *A. sulfonivorans*  $\beta$ -galactosidase, purification and characterization of the enzyme.

### Material and Methods

Fast protein liquid chromatography (chromotograph ACTA FPLC, GE), electrophoretic analysis (**Mini-PROTEAN Tetra Cell**, Bio-Rad), PCR (thermal cycler T100, Bio-Rad), DNA sequencing (LI-COR 4300 DNA Analyzer, LI-COR Biosciences).  $\beta$ -Galactosidase activity was assayed using o-nitrophenyl- $\beta$ -D-galactopyranoside as substrate according to (5). All used chemicals were of analytical grade and commercially available.

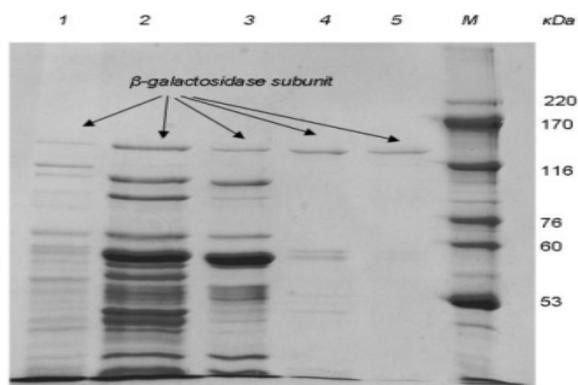
### Results and Discussion

A novel extracellular  $\beta$ -galactosidase from *A. sulfonivorans* was recovered with an overall 207-fold purification, a 7.7% yield and specific activity 16 300 U·mg<sup>-1</sup> protein (**Table 1**).

**Table 1. Purification of  $\beta$ -galactosidase**

Step	Total activity, Ux10 <sup>3</sup>	Total protein, mg	Specific activity, U/mg	Activity yield, %	Purification factor
Crude extract	21,00	270,00	77,77	100	1,00
DEAE-cellulose	14,91	188,40	79,10	71,00	1,02
DEAE-sepharose	5,19	15,90	326,41	24,71	4,19
Superdex 200	4,62	5,00	904,00	22,00	11,62
MonoQ	1,61	0,10	16130,0	7,68	207,40

The subunit molecular mass of the enzyme determined by SDS-PAGE analysis equalled 125 kDa.



**Figure 1.** Crude extract of  $\beta$ -galactosidase (1), concentrate of crude extract (2), elute from DEAE-cellulose (3), DEAE-sepharose (4), Superdex 200 (5), MonoQ (M).

It was found that the enzyme displays pI 5.35, prefers ortho-nitrophenyl- $\beta$ -galactoside as substrate ( $K_m$  27 mM) and shows maximum activity at 40°C and at pH 7.5–9.5

The  $\beta$ -galactosidase gene was isolated from the genomic DNA library of *A. sulfonivorans*, sequenced, cloned and de-

posited in the GenBank database under accession number KM277894.1. It was established that the gene carries an open reading frame consisting of 3132 bp (1043 amino acids) and encodes  $\beta$ -galactosidase referred to Glycosyl Hydrolase Family 2 (CAZy database).

## Conclusion

Potential applications of  $\beta$ -galactosidase include hydrolysis of milk lactose, enzymatic synthesis of galactooligosaccharides *in vivo* and *in vitro* and further use in the formulation of prebiotic feed supplements.

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