Data in Brief 6 (2016) 176–183

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data article

Influence of a family 29 carbohydrate binding module on the recombinant production of galactose oxidase in *Pichia pastoris*

Filip Mollerup [a,*], Emma Master [a,b]

[a] *Department of Biotechnology and Chemical Technology, Aalto University, 00076 Aalto, Finland*
[b] *Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, Ontario,*
Canada M5S 3E5

A R T I C L E I N F O

Article history:
Received 27 October 2015
Accepted 10 November 2015
Available online 26 November 2015

Keywords:
Galactose oxidase
Carbohydrate binding modules
CBM29
Fermentation
Protein production
Protein purification
Enzyme fusion

A B S T R A C T

Herein, we report the extracellular expression of carbohydrate active fusion enzymes in *Pichia pastoris*. Particularly, CBM29-1-2 from *Piromyces equi* was separately fused to the N- and C-terminus of galactose 6-oxidase (GaO, D-galactose: oxygen 6-oxidoreductase, EC 1.1.13.9, CAZy family AA5) from *Fusarium graminearum*, generating CBM29-GaO and GaO-CBM29, respectively. *P. pastoris* was transformed with expression vectors encoding GaO, CBM29-GaO and GaO-CBM29, and the fusion proteins were expressed in both shake-flask and 2L bioreactor systems. Volumetric production yields and specific GaO activity increased when expression was performed in a bioreactor system compared to shake-flask cultivation. This was observed for both CBM29-GaO and GaO-CBM29, and is consistent with previous reports of GaO expression in *P. pastoris* (Spadiut et al., 2010; Anasontzis et al., 2014) [1,2]. Fusion of CBM29 to the C-terminal of GaO (GaO-CBM29) resulted in a stable uniform protein at the expected calculated size (107 kDa) when analyzed with SDS-PAGE. By comparison, the expression of the N-terminal fusion protein (CBM29-GaO) was low, and two truncated versions of CBM29-GaO were coexpressed with the full-sized protein. Despite differences in

DOI of original article: http://dx.doi.org/10.1016/j.bbagen.2015.10.023
* Corresponding author.
E-mail address: filip.mollerup@aalto.fi (F. Mollerup).

http://dx.doi.org/10.1016/j.dib.2015.11.032
2352-3409/© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

protein yield, the specific GaO activity on galactose was not affected by CBM29 fusion to either the N- or C-terminus of the enzyme. A detailed description of the catalytic and physiochemical properties of CBM29-GaO and GaO-CBM29 is available in the parent publication (Mollerup et al., 2015) [3].

© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Specifications Table

Subject area	*Biochemistry and Recombinant Protein Production*
More specific subject area	*Recombinant protein expression of fusion proteins in Pichia Pastoris*
Type of data	*Tables and Figures*
How data was acquired	*Through analysis of data from recombinant protein expression*
Data format	*Data is analysed and presented in text*
Experimental factors	*Recominant expression and purification of fusion proteins constructed by separately appending a family 29 carbohydrate binding module to the N- and C-terminus of galactose oxidase*
Experimental features	*Protein expression in shake-flasks and bioreactor systems and chromatographic methods to purify target proteins from cell culture supernatants*
Data source location	*Not applicable*
Data accessibility	*Data is accessible in this article and upon request to the authors*

Value of the data

1. These results represent the first production and purification study of galactose oxidase fusions to non-native carbohydrate binding modules, and investigates the impact of CBM positioning on protein recovery.
2. To our knowledge, these expression data present the most active preparation of GaO purified from a *P. pastoris* expression host, and simultaneously demonstrate the advantages of using a bioreactor over shake-flask cultivations.
3. Observation of truncated forms of CBM29-GaO, which co-expressed with the full-sized protein. All versions bound efficiently to a Ni-NTA column through a C-terminal His6-tag.
4. Isolation of full-sized CBM29-GaO from its truncated versions by ion-exchange chromatography utilizing slight differences in calculated pI values.

1. Data, experimental design, materials and methods

1.1. Expression of GaO constructs in shake-flasks

The expression vector, fusion protein sequences, and transformation method are reported in Mollerup et al. [3]. *Pichia pastoris* transformants encoding CBM29-GaO or GaO-CBM29 for extracellular expression were grown overnight in 300 mL buffered minimal glycerol medium (BMGY (w/v): 1% yeast extract, 2% peptone, 100 mM potassium phosphate buffer (pH 6.0), 1.34% YNB, 4×10^{-5}% biotin, 1% glycerol) at 30 °C with continuous shaking at 250 rpm. Cells were harvested by centrifugation (1500g) at room temperature and suspended in buffered minimal methanol med-

Download English Version:

https://daneshyari.com/en/article/174972

Download Persian Version:

https://daneshyari.com/article/174972

Daneshyari.com