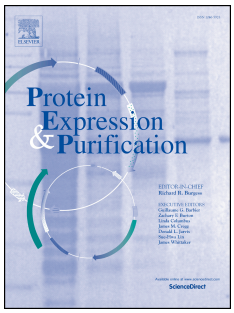
Accepted Manuscript



Heteroexpression and biochemical characterization of a glucose-6-phosphate dehydrogenase from oleaginous yeast *Yarrowia lipolytica*

Mingjie Bian, Shan Li, Huanhuan Wei, Shiping Huang, Feng Zhou, Youming Zhu, Guoping Zhu

PII: S1046-5928(17)30776-3

DOI: 10.1016/j.pep.2018.03.007

Reference: YPREP 5239

To appear in: *Protein Expression and Purification*

Received Date: 31 December 2017

Revised Date: 7 February 2018

Accepted Date: 21 March 2018

Please cite this article as: M. Bian, S. Li, H. Wei, S. Huang, F. Zhou, Y. Zhu, G. Zhu, Heteroexpression and biochemical characterization of a glucose-6-phosphate dehydrogenase from oleaginous yeast *Yarrowia lipolytica*, *Protein Expression and Purification* (2018), doi: 10.1016/j.pep.2018.03.007.

Heteroexpression and biochemical characterization of a glucose-6-phosphate dehydrogenase from oleaginous yeast *Yarrowia lipolytica*

Mingjie Bian[1,†], Shan Li[1,†], Huanhuan Wei[1,†], Shiping Huang[1], Feng Zhou[1], Youming Zhu[2,*], Guoping Zhu[1,*]

[1] Institute of Molecular Biology and Biotechnology and the Research Center of Life Omics and Health, Anhui Normal University, No.1 Beijing East Road, Wuhu 241000, Anhui, China

[2] Department of Oral and Maxillofacial Surgery, School of Stomatology, Stomatological Hospital, Anhui Medical University, No.81 Mei Shan Road, Hefei 230032, Anhui, China

***** Correspondence: gpz2012@ahnu.edu.cn (G.-P.Z.); zym88888@mail.ustc.edu.cn (Y.-M.Z.); Tel: +86-553-3883539; Fax: +86-553-3883592.

[†] These authors contributed equally to this work.

Abstract: *Yarrowia lipolytica*, a nonpathogenic, nonconventional, aerobic and dimorphic yeast, is considered an oleaginous microorganism due to its excellent ability to accumulate large amounts of lipids. Glucose-6-phosphate dehydrogenase (G6PD) is one of two key enzymes involved in the lipid accumulation in this fungi, which catalyzes the oxidative dehydrogenation of glucose-6-phosphate to 6-phosphoglucono-δ-lactone with the reduction of $NADP^+$ to NADPH. In this study, the full-length gene of G6PD from *Y. lipolytica* (*Yl*G6PD) was cloned without intron and heterogeneously expressed in *E. coli*. Then, *Yl*G6PD was purified and biochemically characterized in details. Kinetic analysis showed that *Yl*G6PD was completely dependent on $NADP^+$ and its apparent K_m for $NADP^+$ was 33.3 μM. The optimal pH was 8.5 and the maximum activity was around 47.5 °C. Heat-inactivation profiles revealed that it remained 50% of maximal activity after incubation at 48 °C for 20 min. *Yl*G6PD activity was competitively inhibited by NADPH with a K_i value of 56.04 μM. Most of the metal ions have no effect on activity, but Zn^{2+} was a strong inhibitor. Furthermore, the determinants in the coenzyme specificity of *Yl*G6PD were investigated. Kinetic analysis showed that the single mutant R52D completely lost the ability to utilize $NADP^+$ as its coenzyme, suggesting that Arg-52 plays a decisive role in $NADP^+$ binding in *Yl*G6PD. The identification of *Y. lipolytica* G6PD may provide useful scientific information for metabolic engineering of this yeast as a model for bio-oil production.

Keywords: *Yarrowia lipolytica*; Glucose-6-phosphate dehydrogenase; hetero-expression; biochemical characterization; coenzyme specificity determinants; kinetics

Abbreviations: $NADP^+$, nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NAD^+, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide; PCR, polymerase chain reaction; LB, Luria-Bertani; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; CD,