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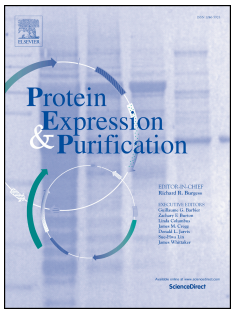

Cloning, overexpression, and purification of glucose-6-phosphate dehydrogenase of *Pseudomonas aeruginosa*

Kevin E. Acero-Navarro, Mariella Jiménez-Ramírez, Miguel A. Villalobos, Rocío Vargas-Martínez, Hugo V. Perales-Vela, Roberto Velasco-García

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Cloning, Overexpression, and Purification of Glucose-6-Phosphate Dehydrogenase of *Pseudomonas aeruginosa*

Kevin E. Acero-Navarro[1], Mariella Jiménez-Ramírez[1], Miguel A. Villalobos [2], Rocío Vargas-Martínez[1], Hugo V. Perales-Vela[3] and Roberto Velasco-García[1,*]

[1] Laboratorio de Osmorregulación, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México. Tlalnepantla, Estado de México, México. C. P. 54090.

[2] Laboratorio de Biología Molecular de Plantas. Centro de Investigación en Biotecnología Aplicada, Instituto Politécnico Nacional. Ex-Hacienda San Juan Molino, Tlaxcala, México.

[3] Laboratorio de Bioquímica, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México. Tlalnepantla, Estado de México, México. C. P. 54090.

*Corresponding author: robertvela2001@yahoo.com.mx; Tel.: 01152-55-56231260

Abstract: Glucose-6-phosphate dehydrogenase (G6PDH) (EC 1.1.1.363) plays an important role in the human pathogen *Pseudomonas aeruginosa* because it generates NADPH, an essential cofactor for several biosynthetic pathways and antioxidant enzymes. *P. aeruginosa* G6PDH is also a key enzyme in the metabolism of various carbon sources, such as glucose, glycerol, fructose, and mannitol. Understanding the kinetic characteristics and mechanisms that control the activity of this enzyme is crucial for future studies in this context. However, one of the impediments to achieving this goal is the limited amount of protein obtained when current purification protocols are implemented, a factor curtailing its biochemical characterization. In this study, we report a fast, efficient and reproducible procedure for the purification of *P. aeruginosa* G6PDH that can be implemented in a short period (2 days). In order to establish this protocol, the *zwf* gene, which encodes for this enzyme, was cloned and overexpressed in *Escherichia coli* cells. In contrast to other procedures, our method is based on protein precipitation with $CaCl_2$ and further purification by ion exchange chromatography. Using this protocol, we were able to obtain 31 mg/l of pure protein that manifested specific activity of 145.7 U/mg. The recombinant enzyme obtained in this study manifested similar physicochemical and kinetic properties to those reported in previous works for this molecule. The large quantities of active enzyme obtained using this procedure will facilitate its structural characterization and identify differences between *P. aeruginosa*- and human G6PDH, thus contributing to the search for selective inhibitors against the bacterial enzyme.

Keywords: *Pseudomonas aeruginosa*, Glucose-6-phosphate dehydrogenase, Entner-Doudoroff pathway, Heterologous overexpression, Cooperative binding.