Detection of soluble expression and *in vivo* interactions of the inner membrane protein OppC using green fluorescent protein

Q.J. Xiang[2,3], J.F. Zhai[1], M. Zhang[1] and B. Zhang[1]

[1]Department of Gynecology, Central Hospital of Xuzhou,
Affiliated Hospital of Southeast University, Xuzhou, China
[2]Department of Microbiology, College of Resource and Environmental Sciences,
Sichuan Agricultural University, Chengdu, China
[3]College of Life Sciences,
Sichuan Key Laboratory of Molecular Biology and Biotechnology,
Sichuan University, Chengdu, China

Corresponding authors: M. Zhang / B. Zhang
E-mail: aimeng860107@126.com / bettyzhang10@163.com

Genet. Mol. Res. 14 (4): 17834-17846 (2015)
Received August 16, 2015
Accepted October 13, 2015
Published December 22, 2015
DOI http://dx.doi.org/10.4238/2015.December.22.8

ABSTRACT. In this study, the *in vivo* interaction system of oligopeptide permease (Opp) proteins was analyzed, and a high expression system of inner membrane protein OppC was constructed by flexible usage of the green fluorescent protein (GFP). The *Escherichia coli OppC* gene, which encodes a transmembrane component of oligopeptide transporter, was cloned into different vectors. Recombinant plasmids were transformed into different *E. coli* strains, and the expression conditions were optimized. The effect of plasmids and expression strains on OppC production was evaluated by in-gel and western blot analyses. OppC produced by the pWaldo-GFPe vector, harboring the GFP reporter gene, transformed into *E. coli* C43(DE3) provided sufficient functional protein for biochemical and biophysical studies. *In vivo* protein-protein interactions were detected among oligopeptide permease proteins using a GFP fragment reassembly

protocol. The substrate binding protein OppA showed no interaction with the other components, while the ATP-binding component OppD did not interact with OppF. OppD and OppF interacted with the transmembrane components OppB and OppC. OppB also showed direct interaction with OppC. *In vivo* OppC functionality was determined by constructing an *OppC* gene deletion strain. OppC was shown to be essential for peptide uptake, and non-essential for cell viability. These results could help in elucidating the oligopeptide transport mechanism in bacteria.

Key words: Oligopeptide permease; Protein-protein interaction; Inner membrane protein; Green fluorescent protein

