Cloning and heterologous expression of Candida albicans SC5314 deoxyhypusine hydrolase gene in E. coli.

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Candida albicans (C. albicans) is a fungal pathogen that causes mucosal and systemic infections in immunocompromised people. Finding both selective and less toxic treatment against C. albicans is required and could be achieved by the regulation of transcription and translation [2]. Hypusination is a post-translational modification of eukaryotic translational factor 5a (eIF5A) in which unusual amino acid, hypusine is formed from a specific lysine residue [3]. This modification involves two enzymes, deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH). At the first stage of hypusination, the aminobutyl fragment of spermidine is transferred to the ε-amino group of lysine by DHS and forms deoxyhypusine, which is then hydroxylated with DOHH to hypusine [1], [3]. This paper is about cloning and expression of DOHH gene in E. coli to obtain protein for biochemical study and crystallization. DOHH gene (Genbank KHC88093.1) was amplified from isolated total Candida albicans DNA by PCR using Tersus polymerase and specific primers (Evrogen, Russia). The resulting gene was ligated with the pETGB1a plasmid vector, and transformed into E. coli DH5a cells. Plasmid DNA (pETGB1a: dohh) was isolated and was further transformed into E. coli BL21 for expression, in LB medium at 37 ° C. Protein synthesis was induced by adding isopropyl-β-D-1-thiogalactopyranoside (IPTG) to a final 0.5 mM, at 18 ° C overnight. BL21 cells were pelleted, resuspended, and lysed. The lysate was centrifuged at 45000 rpm for 45 min. Protein 6xHis-GB1-CaDOHH, was purified by Ni-NTA agarose chromatography. The obtained protein was further purified to CaDOHH 43 kDa. DOHH catalyzes the last hypusination reaction that leads to the activation of eIF5A in all eukaryotes. The resulting construct and the developed protocol for CaDOHH isolation from E. coli make it possible to obtain a purified protein for structural studies.

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