Functional Analysis of GPI-anchored Cell Wall Proteins in Pichia pastoris (Postprint)

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Abstract

Pichia pastoris, as a commonly used protein expression system, is widely applied in laboratory-scale protein preparation, characterization, and structural analysis, with thousands of proteins having been successfully expressed in the Pichia pastoris system. In the field of industrial enzyme preparations, many enzymes including phytase, lipase, mannanase, and xylanase have also achieved industrial-scale production using Pichia pastoris [1].

The yeast cell wall plays a crucial role in protecting cellular integrity, maintaining osmotic pressure balance, generating and maintaining cell morphology during morphogenesis, and protecting cells under environmental stress [2]. The cell wall is primarily composed of polysaccharides and proteins, with the polysaccharides being -1,3-glucan, -1,6-glucan, and chitin, and the proteins mainly consisting of N- and O-modified proteins. Electron microscopy observations have revealed that the yeast cell wall is approximately 200 nm thick and consists of two layers with different electron densities: an outer mannoprotein layer and an inner glucan skeleton [3]. GPI-anchored proteins are a class of proteins that are localized to the cell surface through a glycolipid covalently attached to the C-terminus of the protein. GPI-anchored proteins exist in all eukaryotic cells, and their basic structure is conserved across most organisms. Through analysis of all encoded protein sequences in the Pichia pastoris genome, combined with the structural characteristics of GPI cell wall proteins, 50 potential GPI cell wall proteins were ultimately identified in Pichia pastoris GS115 [4], and further exploration of the biochemical analysis and functional characterization of these proteins is required.

By individually knocking out each of the 50 potential GPI cell wall proteins in Pichia pastoris GS115, a library of GPI cell wall protein-deficient strains was constructed, and the growth and morphological changes of each cell wall-deficient strain under different carbon source concentrations, as well as changes in cell

surface hydrophobicity and tolerance to cell wall-disturbing agents, were investigated. The study revealed that knockout of cell wall proteins induces various cellular changes, such as differences in carbon source utilization and variations in tolerance to different cell wall-disturbing agents. A methanol-tolerant strain was selected for transcriptomic analysis, which revealed that cellular metabolic pathways were significantly altered after knockout, with genes related to the synthesis pathways of various cell wall components and plasma membrane sterol synthesis being significantly upregulated, while some stress pathways were also activated to resist damage from high concentrations of methanol. In-depth investigation of cell wall protein function will provide important reference value for using Pichia pastoris as a host strain for heterologous protein expression.

Full Text

Preamble

Pichia pastoris serves as a widely utilized protein expression system, extensively applied in laboratory-scale protein preparation, characterization, and structural analysis, with over a thousand proteins successfully expressed in this system to date. In the industrial enzyme sector, numerous enzymes including phytase, lipase, mannanase, and xylanase have achieved industrial-scale production using *P. pastoris* [1].

The yeast cell wall plays a crucial role in maintaining cellular integrity, preserving osmotic balance, generating and sustaining cell morphology during morphogenesis, and protecting cells under environmental stress [2]. Composed primarily of polysaccharides and proteins, the cell wall contains -1,3-glucan, -1,6-glucan, and chitin as its main polysaccharide components, while proteins are mainly N- and O-modified species. Electron microscopy reveals that the yeast cell wall is approximately 200 nm thick and consists of two distinct electron-density layers: an outer mannoprotein layer and an inner glucan scaffold [3]. GPI-anchored proteins represent a class of proteins localized to the cell surface through covalent attachment of a glycolipid moiety at their C-terminus. These proteins exist in all eukaryotic cells, and their basic structure is conserved across most organisms. Through genome-wide analysis of all encoded protein sequences in *P. pastoris* combined with structural characteristics of GPI-anchored cell wall proteins, 50 potential GPI-anchored cell wall proteins were identified in *P. pastoris* GS115 [4], though further biochemical analysis and functional characterization of these proteins remain necessary.

By systematically knocking out each of the 50 potential GPI-anchored cell wall proteins in *P. pastoris* GS115, we constructed a comprehensive library of GPI cell wall protein-deficient strains. We investigated the growth characteristics and morphological changes of these mutant strains under various carbon source concentrations, as well as alterations in cell surface hydrophobicity and tolerance to cell wall-disrupting agents. Our findings demonstrate that deletion of cell wall proteins induces multifaceted changes, including differential carbon

source utilization and varying tolerance to cell wall inhibitors. One methanol-tolerant strain was selected for transcriptomic analysis, revealing significant alterations in metabolic pathways upon gene deletion. Notably, genes involved in the synthesis of cell wall components and membrane sterol biosynthesis were substantially upregulated, while several stress response pathways were activated to mitigate damage from high methanol concentrations.

These in-depth functional studies of cell wall proteins will provide valuable insights for optimizing *P. pastoris* as a host for heterologous protein expression.

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Note: Figure translations are in progress. See original paper for figures.