Overexpression of Dihydroxyacetone Synthase 1 in Mut+ *Komagataella phaffii* (*Pichia pastoris*) Improves the Tolerance to Methanol and the Productivity of Recombinant Proteins

Comunicación oral

Biotecnología y bioprocesos

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Komagataella phaffii is a widely used methanol-inducible system for recombinant protein (rProt) production. However, methanol as a sole carbon source reduces the specific growth rate (μ) and promotes intracellular accumulation of formaldehyde, a toxic intermediate. To overcome these limitations and improve rProt productivity, we modified the methanol-assimilative pathway by overexpressing dihydroxyacetone synthase 1 (DAS1).

Three *K. phaffii* GS115 strains were engineered to overexpress DAS1 under different promoters: the strong constitutive glyceraldehyde-3-phosphate dehydrogenase promoter (P_{GAP}), the weak methanol-inducible peroxisomal malate dehydrogenase promoter (P_{MDH3}), and the strong methanol-inducible alcohol oxidase 1 promoter (P_{AOX1}). Each strain expressed Candida antarctica lipase B (CalB) as a model protein. Kinetic characterization was performed in deep-well plates using methanol concentrations ranging from 1 to 20 $g \cdot L^{-1}$. CalB production was assessed in shake-flask and controlled bioreactor cultures.

PAOX1-DAS1 and P_{GAP}-DAS1 strains exhibited higher methanol tolerance, with inhibition constants (Ki) of 52 and 45 $g \cdot L^{-1}$, respectively, compared to the control (Ki =

27 g·L^{-1}). Conversely, P_{MDH3}-DAS1 displayed increased methanol sensitivity (Ki = 17 g·L^{-1}). The maximum specific growth rate (μmax) was highest in P_{GAP}-DAS1 (0.213 h^{-1}). Regarding CalB production, PGAP-DAS1 showed the greatest improvement across all tested conditions, achieving a 21% increase in volumetric productivity (U·L^{-1}·h^{-1}) during batch bioreactor cultivation compared to the control strain.

These findings demonstrate that DAS1 overexpression, particularly under a strong constitutive promoter, enhances methanol tolerance and recombinant protein productivity in *K. phaffii*. This strategy provides a promising approach to improve bioprocess performance when using methanol as both inducer and carbon source.

Palabras Clave

(1) Komagataella phaffii, (2) DAS1, (3) methanol assimilatio, (4) recombinant protein, (5) bioprocess optimization.

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