ID TRABALHO: 204/2667-0

ÁREA DO TRABALHO: MICROBIOLOGIA INDUSTRIAL E BIOTECNOLOGIA

TÍTULO DO TRABALHO: Metabolic Engineering And Process Optimization For The Production Of Ethylene Glycol And Glycolic Acid By Komagataella Phaffii

AUTORES: Thályta Fraga Pacheco, Túlio Marcos G. Andrade, Nathália Aline Torres, Eduardo Brandalise, Lívia Duarte, João Ricardo Moreira De Almeida

INSTITUIÇÃO: Embrapa Agroenergia, Universidade de Brasília

RESUMO:

Abstract

Researchers worldwide have been exploring lignocellulosic biomass as a sustainable alternative to fossil-based raw materials for the production of industrially relevant chemicals. Ethylene glycol (EG) and glycolic acid (GA) are versatile molecules produced by the petrochemical industry and are widely used as chemical monomers or building blocks for synthesizing a broad range of compounds in the chemical and pharmaceutical industries. This study aimed to develop a process for producing ethylene glycol (EG) and glycolic acid (GA) from biomass hydrolysates using robust strains of the yeast Komagataella phaffii. To achieve this, the recombinant strain K. phaffii JA122, capable of producing EG and GA, was constructed by expressing four enzymes (XDH, XD, ALDO, and either ALDR or ALDH). The recombinant strain produced 1.5 g/L of EG and 3.0 g/L of GA. Strain engineering and fermentation process optimization were pursued to optimize EG and AG production. Increasing the expression level of the XD enzyme resulted in a 67% increase in EG production. Given that the EG and GA biosynthetic pathways are competitive, efforts were made to identify the native genes encoding ALDR and ALDH. Specific genes were selected for deletion in the yeast genome. The deletion of one of these genes led to a reduction in GA production from EG, particularly during the first 24 hours of fermentation. To enhance K. phaffii's tolerance to inhibitors present in biomass hydrolysates, genes potentially involved in the yeast's response to such compounds were selected for validation. Five genes were overexpressed in the yeast, and tolerance assays with the recombinant strains against HMF, furfural, and biomass hydrolysates demonstrated that at least three of these genes conferred increased tolerance to the recombinant strains. Finally, several process improvement strategies were employed to optimize aeration conditions, pH, substrate and inoculum concentrations, and other relevant parameters. The results led to more than a 200% increase in EG and GA production and identified aeration as a key factor influencing the biosynthesis of these compounds. In conclusion, this study demonstrates the potential of K. phaffii for producing bio-based chemicals.

Keywords: bioproduct, yeast, metabolic engineering, renewable chemicals, xylose

Acknowledgments: CNPq, FAPDF, CAPES