Chemical Engineering Thesis Defense

Markerless Genome Editing in C. glutamicum Using CRISPR-Cas9.

School for Engineering of Matter, Transport and Energy

Sumant Brahmankar

Advisor: Arul Mozhy Varman

Abstract

Metabolic engineering has emerged as a highly effective approach to optimizing industrial fermentation processes by introducing purposeful genetic alterations using recombinant DNA technology. Successful metabolic engineering begins with a careful investigation of cellular function, and based on the outcomes of this analysis, an improved strain is created and then constructed using genetic engineering. By modifying the genetic makeup of cells, we can increase the production of important chemicals, biofuels, medications, and agricultural products. The most often used genetic engineering tool is plasmid-based gene editing. In plasmid-based gene editing, the desired gene sequence is flanked by similar genome sequences, which encourages the foreign gene's integration into the genome. The main flaw of plasmid-based editing is the presence of selectable markers in the integrated DNA, which impacts cell stability as well as downstream applications that are critical to industries. Recently, with the growth of science, the new gene-editing technology CRISPR (clustered regularly interspaced short palindromic repeat) has revolutionized the field of gene editing. It has been used to incorporate the foreign genes into the genome of the microbial host without any mark and has more efficiency than the plasmid-based gene editing technique. CRISPR is utilized to achieve markerless integration of genes in genomes of microbes, which promotes cell stability and is also especially beneficial for applications in industries. In this experiment, I successfully integrated two genes into the genome of C.glutamicum employing markerless integration via homologous recombination, allowing cells to metabolize acetate into acetyl-CoA and improve the conversion of pyruvate into lactate. Further, this strain of C.glutamicum can be utilized as a platform for producing ethyl lactate, a green solvent using a microbial host.

April 10, 2024; 2:00 PM; BDC.CL1-21

Arizona State University