

Integrated automated synthetic chromosome assembly and transformation platform for high-value co-product expression in a fuel ethanol-producing strain to express a low-calorie protein sweetener as a high-value co-product in an industrial stable *S. cerevisiae* strain engineered to use lignocellulosic or starch sugars for biofuel production

Stephen R. Hughes, Hiep Tran, and Tauseef Butt

USDA, Agricultural Research Service, USA

Several natural proteins extracted from plants have sweet-tasting properties. These plants have been consumed by local populations for centuries, but proteins and genes were isolated only recently. The simplest of these proteins is brazzein, 54-amino-acid monomer, which is 2000 times sweeter than sucrose. Brazzein is remarkably heat-stable and maintains its sweetness even after 4 hours at 350°F. Based on the potential commercial impact of a low-calorie, peptide sweetener with remarkable thermal stability, brazzein was expressed in a stable recombinant ethanologenic industrial yeast strain as a potential high-value co-product in a fuel ethanol operation. Using plasmid-based pSUMO duo vectors, we have identified a set of genes necessary for *Saccharomyces cerevisiae* to use cellulosic sugars and applied automated protocols on an advanced robotic platform to produce pSUMO duo vector cassettes into a stable pYAC4 artificial chromosome expression platform for expression of a polypeptide fusion gene set. High throughput synthetic open reading frame assembly was performed on the synthetic gene assembly and will be used to make 40,000 variant copies of each gene open reading frame placed into pYAC4 as a SUMO cassette that could be mass transformed into the yeast. Stable transformants that grow on pentose sugars under anaerobic conditions will be analyzed for cellulosic ethanol yield and for addition of gene open reading frames for expression of high-value brazzein co-product.

Biography

Stephen Hughes has completed his Ph.D at the age of 25 years from University of Cincinnati Medical College and postdoctoral studies at Hoechst Marion Roussel Pharmaceuticals Inc.. He is a GS-15 Research Molecular Biologist with the United States Department of Agriculture in the Agricultural Research Service at the National Center for Agricultural Utilization Research in Peoria, IL. He has published more than 25 papers in reputed journals and serving as an editorial board member of repute.

stephen.hughes@ARS.USDA.GOV