SL 301

VERY HOT AND ELECTRIFYING MICROBIAL TECHNOLOGIES, J.G. Zeikus, Ph.D., Professor of Biochemistry and Microbiology, Michigan State University and President and CEO, MBI International, 3900 Collins Road, Lansing, MI 48909-0609 USA. We have applied genetic engineering techniques to make improved starch processing enzymes (i.e. thermozymes) from thermophilic and hyperthermophilic microbes. The goal of our research includes identifying the molecular determinants for high stability and activity of ∀-amylase and glucose isomerase (GI). An ∀-amylase gene was cloned from *P. furiosus* that was modified by SD mutagenesis to be three-fold more active and thirteen-fold more stable at 95°C than commercial enzyme from *B. licheniformis*. An essential cysteine disulfide bond was responsible for the formation of a hyper thermostable ∀-amylase dimer in the absence of Ca^{++}. Amylopullulanase genes (APU) were cloned and their respective thermozymes were characterized from *T. ethanolicus* and *P. furiosus*. Nested deletion mutants were used to identify distinct thermal stability and thermal activity regions in the enzyme and gene. The temperature optimum of *T. ethanolicus* APU was shifted from 85 to 65°C without decreasing enzyme thermal stability. GI genes from *T. thermosulfurigenes* (TTXI) and *T. neapolitana* (TNXI) were cloned, expressed and characterized. SD mutants were derived that doubled the specific activity for glucose conversion to fructose by TTXI at 55°C and by TNXI at 97°C. Insertion of proline residues in surface loop regions of GI enhanced both sub-unit interactions and enzyme thermal stability. Fermentation derived succinic acid has many potential specialty chemical and 4-carbon intermediate chemical uses in industry. High succinic acid yields from glucose (110 g/L) were achieved with mutant strains of *Actinobacillus succinogenes*. Regulation of CO_2 fixation and carbon and electron flow in the PEPCK pathway for succinic acid synthesis is described in relation to enhancing product yields and rates. Electrochemical bioreactor systems with electron mediators and end product recovery systems were utilized to enhance the rate and yield of succinate production as well as to lower the cost of end product recovery. During growth of *Actinobacillus* on glucose plus electricity versus on glucose alone, electricity increased glucose consumption, growth, and succinate production by approximately 20% while it decreased acetate production by approximately 50%.