196a Metabolic Engineering of the Xylose Utilizing Thermophile Thermoanaerobacterium Saccharolyticum Jw/Sl-Ys485 for Ethanol Production

A. Joe Shaw IV, R. Lance Martin, Sunil G. Desai, Mike Tyurin, and Lee R. Lynd
Metabolic engineering of end-product metabolism is described with the goal of increasing the yield of
ethanol in place of organic acids (acetic and lactic) produced by wild-type strains. Using targeted gene
knockout in conjunction with a high-frequency gene transfer system, a strain has been created that does
not produce lactic acid. A second strain has also been created that does not produce acetic acid. Both
knockout strains grow at rates comparable to the wild-type. Elimination of acetate production is of
particular note in that this involves significant changes in carbon and electron flux as well as ATP yield.
Our results establish for the first time the feasibility of directing flux to ethanol in lieu of acetic acid
without involvement of pyruvate decarboxylase. Efforts to validate additional selective markers and to
develop and evaluate double knockout strains will be described.