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Sustainable Biofuels Production Through Understanding Fundamental Bacterial Pathways Involved in Biomass Degradation and Sugar Utilization

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Abstract
Genomic analysis and physiological experiments conducted on the lignocellulosic biomass degrading bacterium *C. phytofermentans,* indicates that it can degrade and utilize a wide-range of carbohydrates as possible growth substrates. Previous experiments characterized gene expression using custom whole genome oligonucleotide microarrays. The results indicated that *C. phytofermentans* utilizes ATP-binding cassette (ABC) transporters for carbohydrate uptake and does not use the sole phosphoenolpyruvate-phosphotransferase system (PTS) for any of the tested substrates.Distinct sets of Carbohydrate Active Enzymes (CAZy) genes were also up-regulated on specific substrates indicative of *C. phytofermentans* ability to selectively degrade lignocellulosic biomass. We also identified a highly expressed cluster of genes which includes seven

extracellular glycoside hydrolases and two ABC transporters with unknown specificity on a number of substrates. These results lead to the hypothesis that when grown on plant biomass, *C. phytofermentans*is capable of degrading and transporting all major carbohydrate components of lignocellulose biomass. To test this, *C. phytofermentans* was grown on three different lignocellulosic biomass substrates (*Brachypodium distachyon*, cornstover, and switchgrass). Gene expression and HPLC analysis indicated that *C. phytofermentans* is utilizing multiple substrates with multiple sugar ABC transporter clusters, glycoside hydrolases, and sugar utilization pathways being expressed. To further test this,the sugar utilization pattern for *C. phytofermentans* was investigated. Growth studies were performed on individual saccaharides (glucose, cellobiose, xylose, and fucose) as well as combinations of all these sugars. From these studies we determined that *C. phytofermentans* does not show a characteristic diauxic shift indicative of preferential sugar utilization or carbon catabolite repression (CCR). This result was supported further by HPLC analysis indicating that co-utilization of sugars was occurring, however their were differences in the rates of consumption. Expression analysis of dual sugar combinations of glucose/cellobiose, glucose/xylose, and glucose/fucose also shows that genes involved in the transport and utilization of each sugar are expressed. We also noted glucose repression of some of the glyocside hydrolases which are normally expressed on xylose and fucose. The results from this study indicate that *C. phytofermentans* can utilize multiple sugars simultaneously.

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