## 136. Understanding Fundamental Aspects of Butanol Production by *Clostridium beijerinckii*

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**Project Goals: The solventogenic clostridia offer a sustainable approach to petroleum- based production of n-butanol, an important chemical feedstock and potential fuel. With the availability of the genome sequence for *Clostridium beijerinckii* 8052, we can now employ the tools of systems biology and omics technologies in order to gain increased insights into the metabolic and regulatory networks relevant to solvent production. Project goals include examination of: 1) the mutations underlying the *C. beijerinckii* BA101 butanol-overproduction phenotype, 2) the molecular basis for the global shift from acidogenesis to solventogenesis, 3) the genetic basis of butanol tolerance in *C. beijerinckii* and 4) RNA-seq technology for single-nucleotide resolution analysis of the transcriptome of this microorganism.**

We sequenced four *Clostridium beijerinckii* mutant strains (*C. beijerinckii* BA101, BA105, SA1 and SA2) in combination with our laboratory wild type strain *C. beijerinckii* NCIMB 8052 (UIUC) to examine their genetic differences. Preliminary results show 17 genomic variations (small nucleotide polymorphisms (SNPs), deletions, nucleotide changes, insertions, duplications etc.) in *C. beijerinckii* BA101, 23 genomic variations in *C. beijerinckii* BA105, 15 genomic variations in *C. beijerinckii* SA1 and 55 genomic variations in *C. beijerinckii* SA2. Among these, we discovered two unique polymorphisms for *C. beijerinckii* BA101, seven unique polymorphisms for *C. beijerinckii* BA105 and 23 unique polymorphisms *C. beijerinckii* SA2. Interestingly, *C. beijerinckii* SA1 has no unique genomic variation and shares all 15 alterations with *C. beijerinckii* BA101 and *C. beijerinckii* BA105. These results will lead to specific gene knock-out targets to investigate single genomic variations and their influence on butanol production and/or tolerance.

Based on the mobile group II intron technology, we have developed a Targetron gene knockout system for *C. beijerinckii* (Wang et al., 2013). This system was successfully employed to disrupt acid production pathways in *C. beijerinckii*, leading to *pta* (encoding phosphotransacetylase) and *buk* (encoding butyrate kinase) negative mutants. Compared to the parental strain (*C. beijerinckii* 8052), acetate production in the *pta* mutant was substantially reduced and butyrate production increased. In contrast, acetate and butyrate production in the *buk* mutant was similar to that of the wild type, but solvent production was consistently 20-30% higher and glucose consumption was more rapid and complete. The characterization results suggest that the acid and solvent production of *C. beijerinckii* can be effectively altered by disrupting the acid production pathways. As the gene disruption method we developed does not leave behind an antibiotic marker in the disrupted allele, multiple and high-throughput gene disruptions are possible. Based on this system, we have constructed more than 20 knockout mutants for *C. beijerinckii*, including several double and triple knockout mutants. The characterization of these mutants is currently underway. The results will provide essential information for understanding the basic metabolism of the ABE process in *C. beijerinckii*, and will guide the further improvement of *C. beijerinckii* strains through systems biology and genetic engineering based approaches.

The data collected from metabolic profiling of *C. beijerinckii* 8052 serve to compliment our genome-scale *C. beijerinckii* model (Milne et al., 2011) by adding dynamic information about metabolites in the network in addition to the static stoichiometric equations obtained from known genome-annotations. We observed shifts in metabolism over the course of an 84 hour fermentation, highlighting the transition to saturated fatty acid production to counteract butanol toxicity as butanol concentrations become maximal. Additionally, new information concerning the production and consumption of lactic acid was observed.

We gained additional insight into the physiology of *C. beijerinckii* BA105. This strain demonstrates an acid crash behavior in batch with little butanol being produced when compared to the wild type strain. It accumulates acetate and butyrate and shows significant decreased sporulation; finally, the metabolic switch to acetone-butanol-ethanol (ABE) fermentation does not occur. However, we were able to prevent 'acid-crash' of *C. beijerinckii* BA105 by controlling the fermentation pH. Under controlled conditions, the BA105 strain shows ~50% higher glucose consumption and up to 45% more n-butanol production when compared to the wild type strain. Furthermore, the sporulation capacity of *C. beijerinckii* BA105 is completely restored similar to the wild type. These results lead to the conclusion that *C. beijerinckii* BA105 can be used as a model organism for examining the metabolic switch from acid to solvent production on a genetic and transcriptional level using both non pH-controlled ('acid crash') and pH-controlled (increased butanol production) batch growth experiments.

We also conducted a comprehensive transcriptional analysis using RNA-Seq approach for *C. beijerinckii* BA105 and *C. beijerinckii* 8052 growing on glucose as sole carbon source with pH controlled at 5.5. We examined changes in gene expression in order to evaluate gene targets responsible for higher glucose consumption and higher butanol production. Preliminary results showed significant changes in the phosphotransferase systems (PTS) which are potentially responsible for the increased glucose consumption by *C. beijerinckii* BA105. Furthermore, genes associated with the acid branch (*pta-ack*, *ptb-buk*) demonstrated increased transcript levels and this correlates with the acid accumulation during the growth of the mutant strain. Interestingly, the *sol* operon (*cbei_3832-cbei_3835*) shows decreased transcription in the early growth phase of *C. beijerinckii* BA105, whereas alternative aldehyde-alcohol dehydrogenases are significantly overexpressed, potentially resulting in higher butanol values when compared to the wild type. The combination of acid accumulation and higher butanol titer also led to increased transcript levels of stress genes at the end of the fermentation (e.g., *groES/EL*, *dnaK*). Several sporulation genes were highly repressed in *C. beijerinckii* BA105, although the strain shows restored sporulation when using pH 5.5 controlled conditions.

**References**

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