Genomic-Based Identification of the Sporulation Restoring Gene in Degenerate Clostridium acetobutylicum Strains

There is a renewed interest in the study of Clostridium acetobutylicum due to its applicability in renewable and “greener” production methods for alternative fuels and industrial solvents. Furthermore, due to significant advances in genetic technologies, C. acetobutylicum has ostensibly become a model clostridia for studying other solventogenic and pathogenic clostridia. Of considerable interest are the solventogenesis and sporulation differentiation programs, both of which are abolished in degenerate strains (lacking the 192-kb, 178-gene megaplasmid “pSOL1”). The operon and small genetic locus necessary for solvent formation has been identified and characterized, but the exact gene or operon necessary for sporulation remains a mystery. Knowledge of this gene or operon can be utilized for bioengineering a non-spore forming, solvent producing strain, ideal for industrial continuous fermentations.

To identify this gene or operon, we developed a functional pSOL1 library and selection assay for spore-forming cells. Our approach utilizes multiple libraries of all pSOL1 genes/operons under constitutive promotion in degenerate strains. Our experience suggests that C. acetobutylicum is efficiently transformed by plasmids <10 kb in size, thus library inserts are restricted to 5–6 kb. We created two distinct libraries; a specific PCR generated library and a sheared pSOL1 DNA generated library. To increase cloning efficiency and library versatility, we employed Invitrogen TOPO-TA® cloning and Gateway Technology®. We developed two assays for screening spore-forming cells, one based on chloroform chemical treatment and another by flow-cytometric analysis. We show that chloroform treatment disrupts all C. acetobutylicum cells that have not significantly advanced into endospore formation, such that they are unable to yield colony-forming units when spread onto nutrient plates. Although not yet widely used for prokaryotic analysis, we are developing high-throughput flow cytometry assays capable of discerning single-cell morphology (specifically vegetative versus endospore) based upon membrane potential, DNA content, membrane integrity, and forward/side scatter characteristics. Plasmid DNA is isolated from colonies that survive chloroform treatment and sequenced for the identification of the specific gene/operon.