

Genetic analysis of cellulose degradation by *Clostridium phytofermentans*

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Microbial cellulose degradation is a central part of the global carbon cycle and has potential for the development of inexpensive, carbon neutral biofuels from non-food crops. The major roadblock to the use of cellulosic biomass as a biofuel feedstock is the recalcitrance of cellulosic fibers to breakdown into sugars. *Clostridium phytofermentans* grows on both of the two main components of plant biomass, cellulose and hemicellulose, by secreting enzymes to cleave these polysaccharides and then fermenting the resulting hexose and pentose sugars to ethanol. In order to breakdown cellulose biomass, *C. phytofermentans* has a repertoire of 161 carbohydrate-active enzymes (CAZy), which include 108 glycoside hydrolases spread across 39 families.

Broadly, our goal to understand the genetic mechanisms that permit to *C. phytofermentans* to efficiently convert cellulosic biomass to ethanol. To enable targeted gene inactivation in *C. phytofermentans*, we show that interspecific conjugation with *E. coli* can be used to transfer a plasmid into *C. phytofermentans* that has a resistance marker, an origin of replication that can be selectively lost, and a designed group II intron for efficient, targeted chromosomal insertions without selection. We applied these methods to inactivate Cphy3367, a β -1,4-glucanase in glycoside hydrolase family 9 (GH9). Cellulolytic clostridia usually have numerous genes for GH9 proteins: the *C. thermocellum* ATCC 27405 genome has 16 GH9 genes, *C. cellulolyticum* H10 has 13 GH9 genes, and *C. cellulovorans* has 5 GH9 genes. In contrast, *C. phytofermentans* has only a single GH9-encoding gene, *cphy3367*. The *C. phytofermentans* strain with an intron insertion in *cphy3367* (strain AT02-1) grows normally on some carbon sources such as glucose, cellobiose, and hemicellulose, but has lost the ability to degrade cellulose (Fig 1). Although *C. phytofermentans* up-regulates the expression of numerous enzymes to breakdown cellulose, this process thus relies upon a single, key hydrolase, Cphy3367. Generally, these results show that targeted gene inactivation can be used to identify key enzymes for the breakdown of biomass by *C. phytofermentans*. Future genetic studies of in *C. phytofermentans* will untangle the roles of additional hydrolases in cellulose degradation.

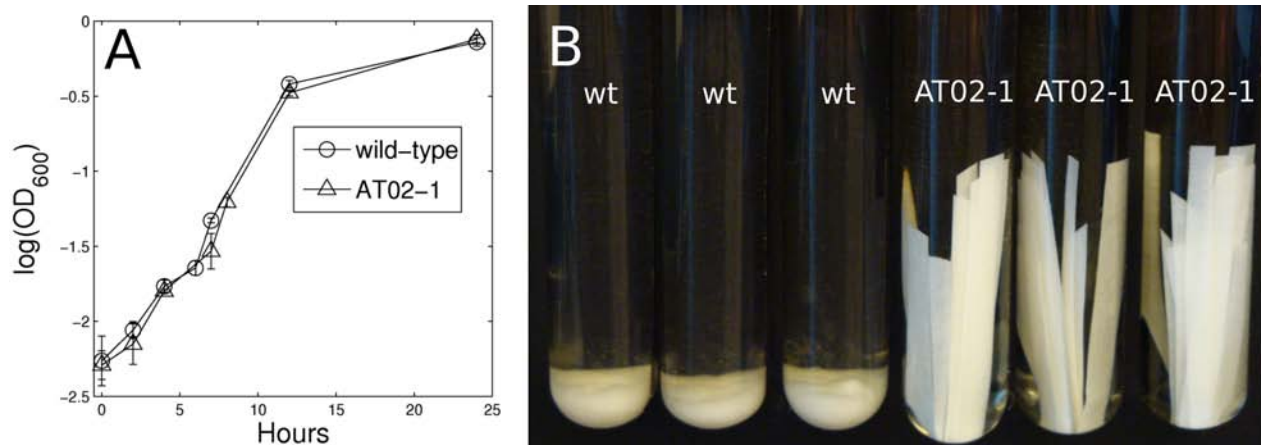


FIG 1 *C. phytofermentans* strain with disruption of *cphy3367* (AT02-1) had similar growth rates as wild-type on glucose **A**, but had lost the ability to degrade filter paper cellulose **B**. Growth curves are means of triplicate cultures. Error bars show one standard deviation and are smaller than the symbols where not apparent.