The Effect of Sugars on Gas Production in *Clostridioides difficile*

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Introduction

- *Clostridioides* (formerly *Clostridium*) *difficile* is an anaerobic, Gram-positive bacterium and is the main causative factor for pseudomembranous colitis.[1]
- The pathogenic strains of *C. difficile* contain a pathogenicity locus (PaLoc) which contains the genes encoding the toxins TcdA and TcdB along with the sigma factor, TcdR.[2]
- The regulation of TcdR, which will subsequently affect toxin production, is influenced by environmental factors, which includes nutrient availability such as glucose and fructose.[2]
- With the known role of glucose in toxin regulation, there may be a role in gas production in *C. difficile*, but this is not completely known.
- One possible pathway of study is the formate hydrogenlyase (FHL) complex, which is utilized in *Escherichia coli* to produce dihydrogen gas and carbon dioxide.[3]
- *E. coli* possesses three isoenzymes of formate dehydrogenase (FDH). Certain FDH in *E. coli* may also contain a selenocysteine residue.[4]
- It is known that *C. difficile* produces dihydrogen gas and possesses genes for FDH containing selenium, however, the possible use of FDH for a FHL complex to produce gas is not entirely known.
- In this study, Durham tubes were used to qualitatively record the differences in gas production between multiple strains of *C. difficile.*
- These strains were grown in Brain Heart Infusion (BHIS) broth supplemented with various sugars to determine if *C. difficile* contains a functional FHL system that is driven by fermentation.
- Wild-type strains R20291 and JIR8094 were tested, along with *selD* mutants to determine if there was any gas production in the *C. difficile* strains that lacked selenium-dependent FDH.

Materials and Methods

To observe the gas production of these strains, six milliliters of BHIS were distributed into screw-cap tubes with Durham tubes and autoclaved. Each sugar was prepared as a 20% stock solution by dissolving two grams of glucose, ribose, fructose, arabinose, trehalose, and xylose in ten milliliters of deionized water and filter-sterilizing each mixture. For each strain, the experiment was run in triplicate for each sugar. Once the stock solutions were sterilized, 300 µL of each sugar were added to their respective screw-cap tubes with BHIS broth. In the anaerobic chamber, overnight cultures were prepared for the *C. difficile* strains R20291 and JIR8094 along with several genetic mutants of each strain. Specifically, these mutants included, with respect to R20291 (wild-type), KNM6 (*ΔselD*) and KNM9 (*ΔselD::selD*$^+$) and, with respect to JIR8094 (wild-type), LB-CD4 (*prdB::ermB*), LB-CD7 (*selD::ermB*), and LB-CD12 (*grdA::ermB*). The starter cultures were incubated overnight at 37 °C. The following day, 120 µL of each overnight culture were inoculated into its respective screw-cap tubes with sugar resulting in a 1:50 dilution for each tube. Each strain had three tubes that did not have a sugar present and therefore served as a negative control. Pictures were taken for two days after the initial inoculation of the wild-type strains into the screw-cap tubes (Figure 1).

On day 2, the Durham tubes were scored on a +/- system to qualitatively represent how much gas was present. A minus indicates that there was no gas production, "+" indicates that the gas filled up one-third of the Durham tube, "++" means that the gas filled about two-thirds of the Durham tube, and "+++" shows that the gas filled up beyond two-thirds of the Durham tube (Tables 1 and 2).

Data

Table 1. Number of tubes based on gas production scoring on day 2 for *C. difficile* strains JIR8094, LB-CD7, LB-CD7, and LB-CD12

Sugar	*C. difficile* Strain															
	JIR8094				LB-CD4				LB-CD7				LB-CD12			
	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++
Control	0	12	0	0	0	3	0	0	1	8	0	0	0	3	0	0
Arabinose	0	9	0	0	0	0	0	0	0	6	0	0	0	0	0	0
Trehalose	0	8	1	0	0	0	0	0	0	3	2	1	0	0	0	0
Fructose	0	7	4	1	0	3	0	0	0	4	5	0	0	3	0	0
Xylose	0	9	0	0	0	0	0	0	1	5	0	0	0	0	0	0
Glucose	0	8	1	0	0	0	0	0	0	6	0	0	0	0	0	0
Ribose	0	9	0	0	0	0	0	0	1	5	0	0	0	0	0	0

Table 2. Number of tubes based on gas production scoring on day 2 for *C. difficile* strains R20291, KNM6, and KNM9

Sugar	*C. difficile* Strain											
	R20291				KNM6				KNM9			
	-	+	++	+++	-	+	++	+++	-	+	++	+++
Control	0	9	0	0	0	6	0	0	0	6	0	0
Arabinose	0	6	0	0	0	3	0	0	0	3	0	0
Trehalose	0	5	1	0	0	3	0	0	0	3	0	0
Fructose	0	9	0	0	0	6	0	0	0	4	1	1
Xylose	0	5	0	0	0	3	0	0	0	3	0	0
Glucose	0	6	0	0	0	3	0	0	0	3	0	0
Ribose	0	6	0	0	0	3	0	0	0	3	0	0

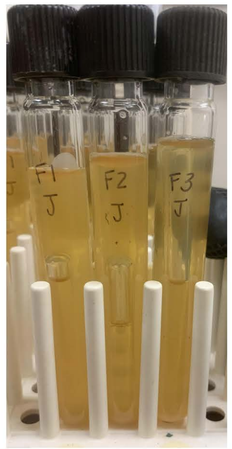

Figure 1. Example of gas production using strain JIR8094 cultured with fructose

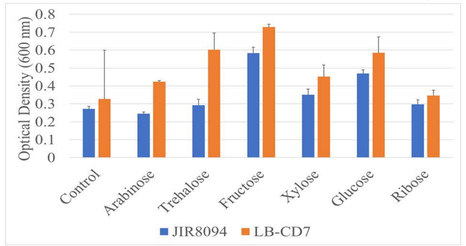

Figure 2. Average optical densities of JIR8094 and LB-CD7 *C. difficile* strains in various sugars on day 2

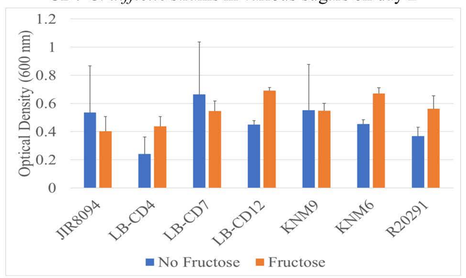

Figure 3. Average optical densities of *C. difficile* strains with or without fructose on day 2

Results

- Figure 1 represents tubes containing the JIR8094 strain with fructose that were scored "+", "++", and "+++" from left to right.
- Table 1 and Table 2 show how many tubes fell into each scoring category based on the *C. difficile* strain and the sugar supplemented in the tube.
- The JIR8094 and LB-CD7 strains that were supplemented with fructose in Table 1 had 4 and 5 tubes respectively where gas filled around two-thirds of the Durham tube.
- One experiment conducted only included *C. difficile* strains JIR8094 and LB-CD7. On day 2 of observing the gas production, an optical density scan was done to estimate how many *C. difficile* cells were present. The results from the optical density scan are present in Figure 2. The wells supplemented with fructose were the ones with the highest estimate for both strains.
- Based on the results from Figure 2, an experiment was conducted where all available *C. difficile* strains were supplemented with only fructose. An optical density scan was conducted for these tubes and the results are present in Figure 3.

Discussion

According to the results, all *C. difficile* strains tested showed gas production with every tested sugar. The few exceptions for this observation included tubes containing LB-CD7 with xylose, ribose, and no sugar (control). Regarding the tubes with significant gas production containing JIR8094 and LB-CD7 in fructose, the first experiment showed that the tubes supplemented with fructose had the greatest gas production. These results were also enforced by the measurements from Figure 2, as both JIR8094 and LB-CD7 had the highest average optical densities when accompanied by fructose. To test for any significance between fructose and gas production in *C. difficile*, all available strains were supplemented with or without fructose. However, the results from this follow-up experiment only resulted in KNM9 having significant gas production. Although there was significant gas production in this strain, the average optical densities of KNM9 in Figure 3 showed no difference in the presence of fructose.

Despite the knowledge that the FDH in *C. difficile* is selenium-dependent, the *selD* mutants, KNM6, KNM9, and LB-CD7, still had gas production for each supplemented sugar. Since the lack of selenium did not affect the gas production of these strains, this may indicate that FDH was utilized in a *selD*-independent pathway, or that it was not used in gas production for *C. difficile*. As FDH is part of the FHL complex, this could show that *C. difficile* may use another pathway for the production of gas.

References

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