# Development of a Probiotic Treatment for *C. difficile* Infections Using Hybrid Microbes That Act Via Multiple Modes of Action

*Authors: Anthony Cann[1], Jeanette Mucha[1], Derik Twomey[1], and Gregory Govoni[1]*

*Affiliation: [1]SciBac Inc., San Francisco, California, USA.*

**Contact info: Greg.Govoni@scibac.com • 650.689.5343 • www.SciBac.com**

## BACKGROUND & AIMS

New antibacterial therapies are urgently needed for antibiotic-resistant and difficult-to-treat bacteria. One such bacterial species, *C. difficile,* is able to exploit disruptions to the gut microbiota in order to proliferate and cause disease. Due to ineffective treatment options, CDI recurrence rates are upwards of 25%. We propose using our patented gene transfer technology to create hybrid organisms capable of combatting CDI and CDI recurrence. Our first aim is to create hybrid organisms capable of growing in nutrient restricted environments such as the inflamed gut while also demonstrating anti-*C. difficile* properties.

## METHODS

The DRIVE (Directed Recombination by *In Vitro* Evolution) gene transfer technology platform harnesses natural horizontal gene transfer to rapidly combine genetic traits from two species into a single hybrid organism. Resulting bacterial hybrids were tested for growth on a carbohydrate panel and minimal media. They were also tested for their effects on 1) *C. difficile* cell viability 2) *C. difficile* adhesion to Caco-2 colon cells and 3) spore viability as measured by CFU. Hybrid yeast strains were tested for anti-toxin activity using an ELISA for *C. difficile* toxins A and B.

## RESULTS

**Figure 1.** Selection of chimeric hybrids via the DRIVE process. Top conditions select against *C. beijerinckii* (high temperature and oxygen environment) and the bottom conditions select against *L. plantarum* (low nutrients).

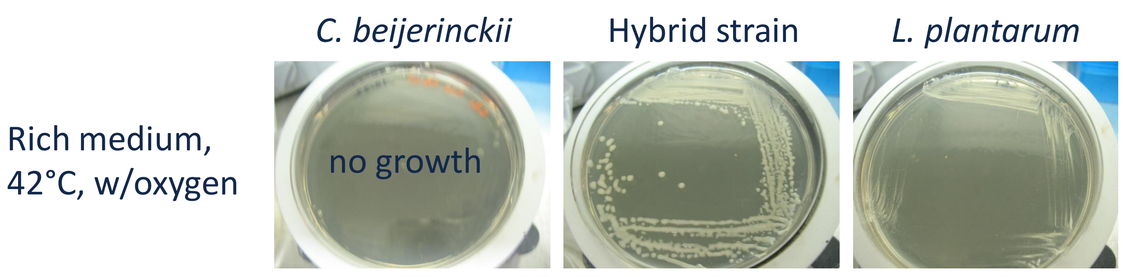
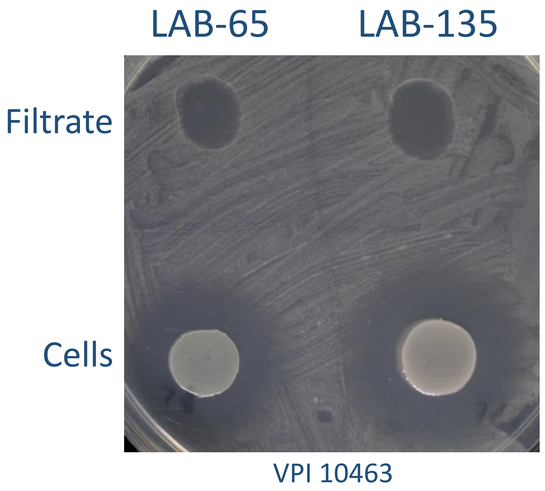



**Figure 2.** Chimeric hybrids demonstrate improved carbohydrate uptake traits when compared to their parents. The hybrid strain shown has the ability to grow on all carbohydrate sources associated with *L. plantarum*, except for ribose (RIB), while also gaining the ability to grow on 6 carbohydrates unique to *C. beijerinckii.*

| BioM CH50 #: | 4 | 5 | 6 | 9 | 10 | 11 | 12 | 13 | 15 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Substrate: | L-ARA | RIB | L-XYL | MDX | GAL | GLU | FRU | MNE | RHA | INO | MAN | SOR | MDM | MDG | NAG | AMY |
| *C. beijerinckii* | | | | | | | | | | | | | | | | |
| Hybrid Strain | | | | | | | | | | | | | | | | |
| *L. plantarum* | | | | | | | | | | | | | | | | |

| BioM CH50 #: | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 39 | 40 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Substrate: | ARB | ESC | SAL | CEL | MAL | LAC | MEL | SAC | TRE | INU | MLZ | RAF | AMD | GLYG | GEN | TUR |
| *C. beijerinckii* | | | | | | | | | | | | | | | | |
| Hybrid Strain | | | | | | | | | | | | | | | | |
| *L. plantarum* | | | | | | | | | | | | | | | | |

**Figure 3.** Chimeric hybrids LAB-65 and LAB-135 inhibit growth of *C. difficile* isolates. A panel of 22 isolates representing a range of common ribotypes was screened for sensitivity.




| Strain | Ribotype | LAB-65 | LAB-135 |
|---|---|---|---|
| 19103 | 1 | ++ | ++ |
| 19099 | 2 | ++ | ++ |
| BAA-1801 | 10 | + | + |
| CD630 | 12 | ++ | ++ |
| 19123 | 14 | + | + |
| TL174 | 15 | ++ | ++ |
| ATCC43598 | 17 | ++ | ++ |
| BAA-1812 | 24 | + | + |
| BAA-1803 | 27 | + | + |
| BAA-1870 | 27 | ++ | ++ |
| 19129 | 27 | ++ | ++ |
| 20068 | 27 | + | + |
| CD196 | 27 | ++ | ++ |
| R20291 | 27 | ++ | ++ |
| BAA-1804 | 53 | + | + |
| BAA-1875 | 78 | + | ++ |
| 8119 | 78 | ++ | ++ |
| 8905 | 78 | ++ | ++ |
| M120 | 78 | ++ | ++ |
| VPI 10463 | 87 | ++ | ++ |
| 19102 | 106 | + | + |
| BAA-1814 | 251 | + | + |

Powered by CARB-X

**Figure 4.** Co-culture of the hybrid organism LAB-65 and LAB-135 inhibit *C. difficile* growth.

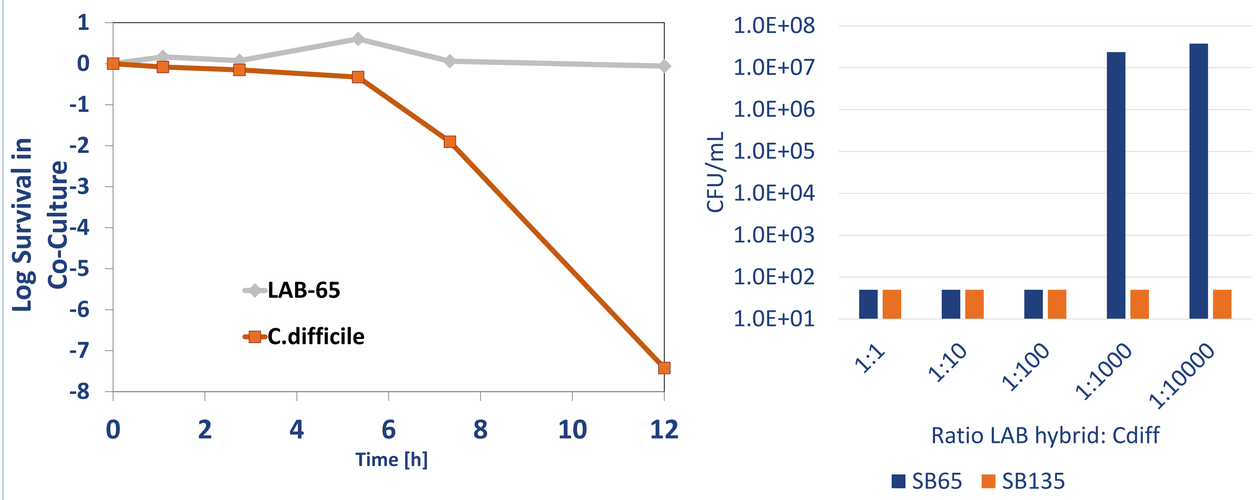



**Figure 5.** The hybrid LAB-65 prevents adherence of *C. difficile* strain VPI10463 to human Caco-2 colon cells. Methods modified from Banerjee *et al*., **Gut Pathogens** 2009.

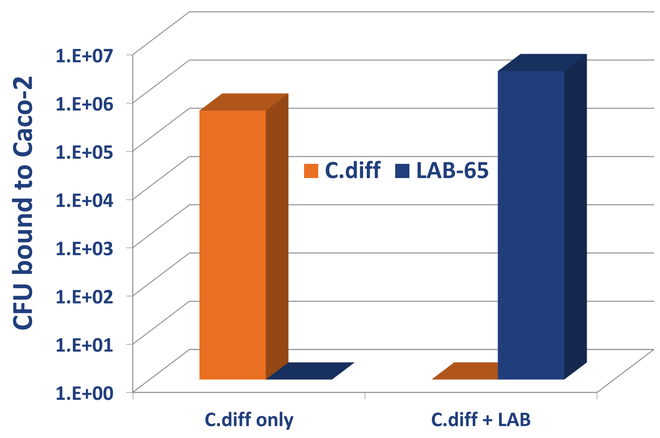



**Figure 6.** The hybrid LAB-65 suppresses spore formation of *C. difficile* strain R20291 when co-cultured in BHIS media.

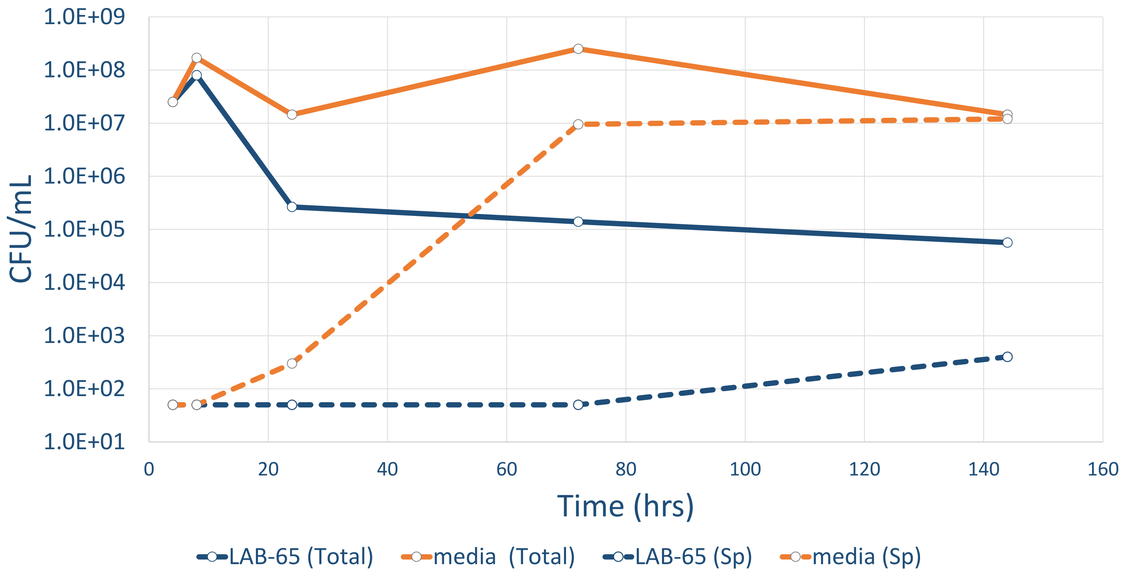
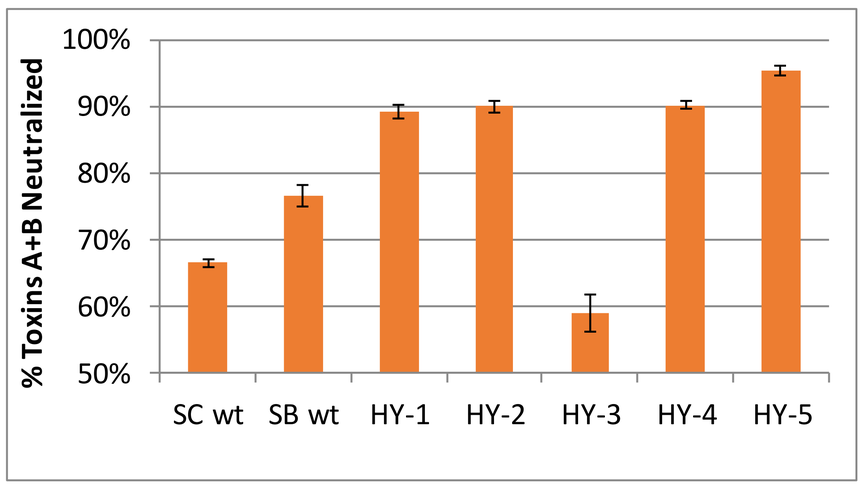



**Figure 7.** Hybrid yeast (HY) strains created using the DRIVE process demonstrate increased anti-toxin activity by ELISA when compared to their *S. boulardii* parent.




## Conclusions

Most treatments for CDI are designed to address a single action of *C. difficile* during infection. By creating multiple hybrid organisms via the DRIVE platform, we were able to create a cocktail of hybrid organisms capable of treating CDI with multiple modes of action while being better suited to withstand inflamed gut environments. This multi-factorial approach is more likely to deliver lasting, successful treatment outcomes when it comes to CDI and CDI recurrences.