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# Quantitative Real Time PCR Detection of In Vitro Clostridium Difficile Growth Inhibition by Popular Over-the-Counter Probiotic Supplements

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## Introduction:

Probiotics are known beneficial or commensal microorganisms. They are often recommended as adjuncts to treatments for antibiotic associated diarrhea (AAD) <sup>1</sup>. It is theorized that supplementation with probiotic organisms competes against pathogenic organisms that colonize the GI tract and cause diarrhea. The aim of this study is to compare the inhibition of *Clostridium difficile* (C diff), a common cause of AAD, by different commercial probiotic products.

## Disclosures:

Ella Dao and Bryan Tran are the founders of BioProper Labs which markets Nexabiotic® and is sponsoring this study. They have a financial interest in the company.

## Design:

Growth of C diff species under anaerobic conditions will be measured via qRT-PCR. Growth rates will be compared between cultures of C diff grown with probiotics and without.

## Hypothesis:

Previous studies <sup>2</sup> by Dr. Larsen found that kefir, a yogurt product, consisting of multiple strains of probiotics demonstrated greater potency of *Clostridium difficile* growth inhibition compared to single strains of probiotics alone. We hypothesize that a multi-probiotic would yield greater inhibition due to greater competition.

## Method:

C diff bacterium will be grown anaerobically and then mixed with dilutions of over the counter probiotic organisms as well as a saline control. The mixtures will be incubated anaerobically for a period of 48 hours and then analyzed via real time quantitative PCR. Cycle threshold numbers for the various mixtures will then be compared to determine the level of growth inhibition.

# Quantitative real time PCR detection of in vitro *Clostridium difficile* growth inhibition by popular over-the-counter probiotic supplements

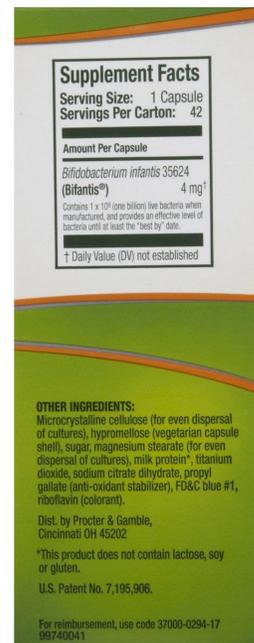
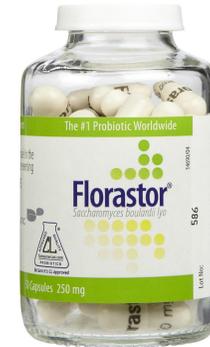
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VS



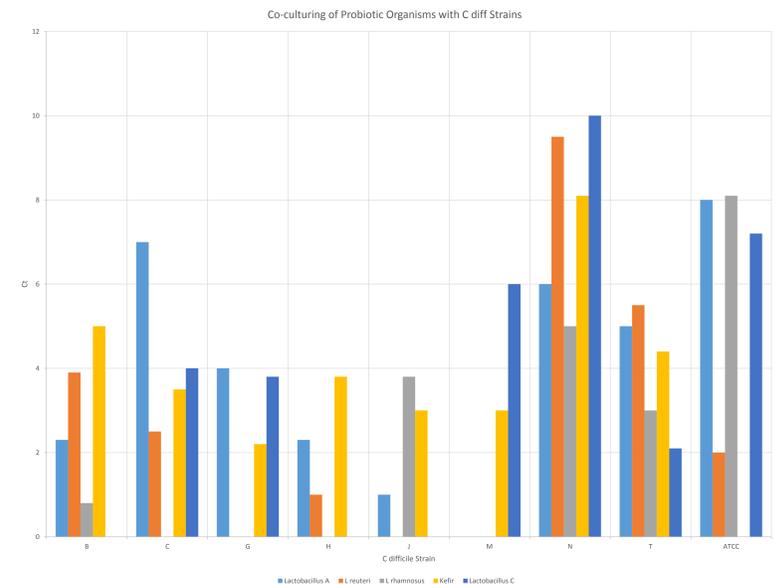
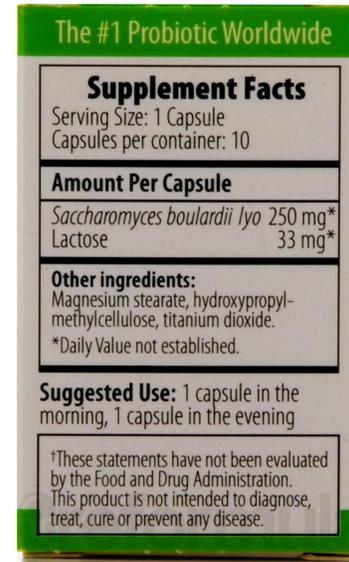
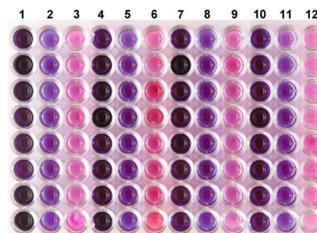
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Supplement Facts	
Serving Size: 2 Vegetarian Capsules	
Amount Per Serving	
<b>23-Strain Probiotic Blend:</b>	<b>34.5 Billion CFUs†*</b>
Saccharomyces Boulardii	
Streptococcus Thermophilus	
Lactobacillus delbrueckii LE	
Lactobacillus rhamnosus LB3	
Lactobacillus plantarum LM	
Lactobacillus acidophilus	
Enterococcus faecium	
Lactobacillus casei	
Lactobacillus helveticus	
Lactobacillus plantarum	
Lactobacillus rhamnosus	
Lactobacillus salivarius	
Lactobacillus lactis	
Lactobacillus paracasei	
Lactobacillus brevis	
Lactobacillus gasseri	
Bifidobacterium bifidum	
Bifidobacterium breve	
Bacillus coagulans	
Bifidobacterium lactis	
Bifidobacterium animalis lactis (formerly named b. infantis)	
Bifidobacterium longum	
Bacillus subtilis	
†Daily value not established.	

Other ingredients: Hypromellose, gellan gum, maltodextrin and medium chain triglycerides oil.

† Potency at time of manufacture.



<sup>2</sup> Co-culture results of the full panel of probiotic organisms or mixtures of organisms (from kefir) indicated by different symbols, against the full panel of 9 strains of *C. difficile* indicated by labels on the x axis. In this graph, inhibition is illustrated by Ct (y axis) which means the difference between the Ct value for qPCR of *C. difficile* alone versus *C. difficile* cultivated in the presence of the probiotic organism(s). Ct values appearing on the baseline indicate that *C. difficile* alone showed equal or lower Ct than that obtained from *C. difficile* co-cultured with the indicated probiotic organism(s) and as Ct values above 2 were considered indicative of a probiotic effect, virtually all potential probiotic organisms showed some activity against *C. difficile*.

## Projected results:

We expect to see greater C diff growth inhibition with the multi-species probiotic Nexabiotic® compared to the single species products Florastor® and Align®. Higher cycle threshold numbers would mean that it took more PCR cycles to amplify the C diff organisms in each culture to the same amount.

## Discussion:

While this study could produce interesting results, a future study could be done in vivo comparing the recovery rates of C diff infected individuals on antibiotics alone versus antibiotics plus name brand probiotics.

## Works Cited:

<sup>1</sup> Hempel, S et al. Probiotics for the Prevention and Treatment of Antibiotic-Associated Diarrhea. JAMA. 2012;307(18):1959-1969. doi:10.1001/jama.2012.3507.

<sup>2</sup> Folkers BL1, Schuring C, Essmann M, Larsen B. Quantitative real time PCR detection of *Clostridium difficile* growth inhibition by probiotic organisms. N Am J Med Sci. 2010 Jan;2(1):5-10. doi: 10.4297/najms.2010.15.