AMERICAN SOCIETY FOR MICROBIOLOGY | mSphere®

COMMENTARY
Host-Microbe Biology

Dilution as a Solution: Targeting Microbial Populations with a Simplified Dilution Strategy

Disha Bhattacharjee,[a] Anna M. Seekatz[a]

[a]Department of Biological Sciences, Clemson University, Clemson, South Carolina, USA

ABSTRACT The gut microbiota is an integral part of maintaining resistance against infection by *Clostridioides* (*Clostridium*) *difficile*, a pathogen of increasing concern in both health care and community settings. The recent article by J. M. Auchtung, E. C. Preisner, J. Collins, A. I. Lerma, and R. A. Britton (mSphere 5:e00387-20, 2020, https://doi.org/10.1128/mSphere.00387-20) demonstrates an innovative approach to identify microbes that inhibit *C. difficile* by employing a dilution scheme to test different microbial mixtures *in vitro* and *in vivo*. This type of approach can advance the identification and validation of specific microbes that elicit functions of interest for many conditions involving the microbiota, of which the complexity and variability can often complicate causality.

KEYWORDS *C. difficile*, FMT, microbiota, minibioreactors

The development of microbial therapeutics to target infections and other health conditions has surged in the last decade. One microbial application, fecal microbiota transplantation (FMT), has received particular attention due to its high rate of success in treating *Clostridioides* (*Clostridium*) *difficile* infection (CDI), an important health care-acquired infection. Because the use of undefined concoctions of fecal material may contain harmful bacterial, viral, fungal, and other components that influence health downstream, strategies to identify a targeted microbial population to treat CDI are of interest. However, both inherent variability in successfully used microbial communities and the ability to rapidly screen these diverse microbes have hindered these efforts. A recent study by Auchtung et al. developed an experimental platform to define bacterial components in the human microbiota that may elicit protection against *C. difficile*, combining *in vitro* and *in vivo* approaches to simplify these complex communities (1). Although that study focuses on CDI, the same approach can be applied to other gastrointestinal diseases influenced by the gut microbiota.

Auchtung et al. first applied a dilution scheme to six fecal samples collected from healthy human participants to generate multiple simplified microbial communities. Multiple diluted communities (at 10^{-4} and 10^{-5} dilutions) were individually seeded into minibioreactor arrays, an approach that has been previously used to study microbiota functions (2). Minibioreactor cultures were then tested for the ability to inhibit *C. difficile* spores. Of these, approximately a third of the simplified communities were able to inhibit *C. difficile* growth by at least 10^4. The use of 16S rRNA gene-based sequencing revealed that dilution decreased the total number of operational taxonomic units (OTUs) in the simplified communities resistant to *C. difficile* from 62 to 42, with each dilution composed of taxonomically diverse microbes that approximated their donor origin. The investigators then chose a subset of microbial mixtures to test their abilities to inhibit *C. difficile* in a mouse model of disease. Mice with an established "humanized" microbiota (3) were pretreated with antibiotics, followed by gavage with one of five simplified, resistant communities and *C. difficile* challenge. Two of the five communities

Citation Bhattacharjee D, Seekatz AM. 2020. Dilution as a solution: targeting microbial populations with a simplified dilution strategy. mSphere 5:e00701-20. https://doi.org/10.1128/mSphere.00701-20.

Address correspondence to Anna M. Seekatz, aseekat@clemson.edu.

For the article discussed, see https://doi.org/10.1128/mSphere.00387-20.

@Annaerobe: Dilution as a solution: check out our comments on a new study by Auchtung et al. that highlights how simplified microbial mixtures can be used to target microbes of interest

Published 29 July 2020

tested demonstrated rapid clearance of *C. difficile*, both following initial challenge and later with relapse. Notably, mice that cleared *C. difficile* also maintained higher body weight than mice that continued to shed *C. difficile*, indicating protection from disease. One of the selected resistant communities that was further diluted (to 28 and 9 OTUs) continued to restrict *C. difficile* expansion. Interestingly, the majority of the organisms in these samples were classified as *Clostridiales*. However, microbial recovery in animals with CDI that were treated with simplified communities was not restricted to organisms found in the input; in fact, OTU tracking suggested that OTUs present in the mouse prior to antibiotic treatment were also significantly enriched. These included OTUs belonging to multiple phyla, such as *Bacteroides*, *Parabacteroides*, E*rysipelotrichaceae*, *Bifidobacterium*, and *Porphyromonadaceae* OTUs.

These data suggest two important points concerning the identification of strains that could ultimately provide support for targeted microbial treatments against CDI: (i) a diverse set of microbes is likely able to inhibit *C. difficile* and (ii) microbes introduced to the system are only part of the recovery process. Much of the focus of CDI therapeutics has focused on identifying microbes that provide functions known to target *C. difficile* itself, such as the production of secondary bile acids that inhibit *C. difficile* growth (4). Other groups have focused on groups of bacteria previously associated with recovery of the microbiota, such as spore-forming bacteria, which include *Clostridiales* (5, 6). Yet data suggest that defining the magic bullet against *C. difficile* is perhaps not as stringent. Previous studies have demonstrated that communities of microbes without the obvious capability to produce secondary bile acids are sufficient to clear *C. difficile* in mice (7). Notably, the simplified communities identified as inhibitory to *C. difficile* did not necessarily harbor any one group of microbes but contained a spectrum of organisms. Instead, as demonstrated in the mice that recovered from CDI following successful microbial intervention, the microbial input may serve as a mechanism to induce expansion of extant host microbiota that eventually inhibit *C. difficile*. This has been demonstrated to some extent in human FMT studies (8, 9). How the host and its microbes "accept" these microbes may be key to understanding how to modulate this environment.

Cultivation of microbes of interest remains an obstacle to development of targeted microbial therapeutics, for CDI and otherwise. The methodology employed by the authors provides a potential solution to isolation, screening, and validation of complex microbial communities, which are often laborious. While this study is not the first to use a dilution/extinction strategy to simplify communities (10) or bioreactors (11), the combination of *in vitro* and *in vivo* approaches to screen simplified communities for a particular output is efficient and easily adapted for other uses. *In vitro* prescreening of defined microbiota mixtures reduces the number of animals necessary for testing multiple combinations of microbes. The approach is also highly applicable for the identification of microbial communities important in other conditions where the microbiota is assumed to be involved. Minibioreactors for identification, screening, and validation of defined microbial populations could easily be modified to look for specific output, such as production of specific metabolites under different conditions or the impact of environmental disruptions on the community function. Indeed, a logical inclusion to this study would have been to use the bioreactor system to identify whether metabolites known to impact *C. difficile* were produced by the simplified communities. This additional measurement may have identified shared functions of seemingly diverse microbial mixtures that influence *C. difficile* physiology, as well as provide some context for how well these functions are recapitulated *in vivo*.

In summary, Auchtung et al. demonstrate a novel approach to identify distinct microbes of interest. As interest in the development of microbial therapeutics to treat disease and maintain health continues, efficient systems as described in that study can advance the process of understanding these complex microbial communities.

REFERENCES

1. Auchtung JM, Preisner EC, Collins J, Lerma AI, Britton RA. 2020. Identification of simplified microbial communities that inhibit *Clostridioides difficile* infection through dilution/extinction. mSphere 5:e00387-20. https://doi.org/10.1128/mSphere.00387-20.
2. Auchtung JM, Robinson CD, Britton RA. 2015. Cultivation of stable, reproducible microbial communities from different fecal donors using minibioreactor arrays (MBRAs). Microbiome 3:42. https://doi.org/10.1186/s40168-015-0106-5.
3. Collins J, Auchtung JM, Schaefer L, Eaton KA, Britton RA. 2015. Humanized microbiota mice as a model of recurrent Clostridium difficile disease. Microbiome 3:35. https://doi.org/10.1186/s40168-015-0097-2.
4. Buffie CG, Bucci V, Stein RR, Mckenney PT, Ling L, Gobourne A, No D, Liu H, Kinnebrew M, Viale A, Jenq RR, Taur Y, Sander C, Cross J, Toussaint NC, Xavier JB, Pamer EG, Service D, Sloan M, Cancer K, Sloan M, Cancer K, Service MT, Sloan M, Cancer K, Programs B, Laboratories MC, Marron CC, Metabolism C, Dartmouth N. 2015. Precision microbiome restoration of bile acid-mediated resistance to Clostridium difficile. Nature 517:205–208. https://doi.org/10.1038/nature13828.
5. Khanna S, Pardi DS, Kelly CR, Kraft CS, Dhere T, Henn MR, Lombardo MJ, Vulic M, Ohsumi T, Winkler J, Pindar C, McGovern BH, Pomerantz RJ, Aunins JG, Cook DN, Hohmann EL. 2016. A novel microbiome therapeutic increases gut microbial diversity and prevents recurrent Clostridium difficile infection. J Infect Dis 214:173–181. https://doi.org/10.1093/infdis/jiv766.
6. Petrof EO, Gloor GB, Vanner SJ, Weese SJ, Carter D, Daigneault MC, Brown EM, Schroeter K, Allen-Vercoe E. 2013. Stool substitute transplant therapy for the eradication of Clostridium difficile infection: "RePOOPulating" the gut. Microbiome 1:3. https://doi.org/10.1186/2049-2618-1-3.
7. Lawley TD, Clare S, Walker AW, Stares MD, Connor TR, Raisen C, Goulding D, Rad R, Schreiber F, Brandt C, Deakin LJ, Pickard DJ, Duncan SH, Flint HJ, Clark TG, Parkhill J, Dougan G. 2012. Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing Clostridium difficile disease in mice. PLoS Pathog 8:e1002995. https://doi.org/10.1371/journal.ppat.1002995.
8. Smillie CS, Sauk J, Gevers D, Friedman J, Sung J, Youngster I, Hohmann EL, Staley C, Khoruts A, Sadowsky MJ, Allegretti JR, Smith MB, Xavier RJ, Alm EJ. 2018. Strain tracking reveals the determinants of bacterial engraftment in the human gut following fecal microbiota transplantation. Cell Host Microbe 23:229–240.e5. https://doi.org/10.1016/j.chom.2018.01.003.
9. Staley C, Kaiser T, Vaughn BP, Graiziger C, Hamilton MJ, Kabage AJ, Khoruts A, Sadowsky MJ. 2019. Durable long-term bacterial engraftment following encapsulated fecal microbiota transplantation to treat Clostridium difficile infection. mBio 10:e01586-19. https://doi.org/10.1128/mBio.01586-19.
10. Garland JL, Lehman RM. 1999. Dilution/extinction of community phenotypic characters to estimate relative structural diversity in mixed communities. FEMS Microbiol Ecol 30:333–343. https://doi.org/10.1111/j.1574-6941.1999.tb00661.x.
11. Carlucci C, Jones CS, Oliphant K, Yen S, Daigneault M, Carriero C, Robinson A, Petrof EO, Weese JS, Allen-Vercoe E. 2019. Effects of defined gut microbial ecosystem components on virulence determinants of Clostridioides difficile. Sci Rep 9:885. https://doi.org/10.1038/s41598-018-37547-x.