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14. ABSTRACT

The current project will analyze the gut microbiome and metabolites from PD patients and controls, and employ clinically relevant mouse models to determine how metabolites produced by the microbiome from dietary substrates affect motor symptoms. We propose to test whether directly regulating microbial metabolite profiles using "designer" dietary fibers and probiotics offers new avenues for ameliorating PD-like symptoms.

15. SUBJECT TERMS

Parkinson's disease, human subjects, intestinal microbiome, stool specimens, gut-brain axis

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INTRODUCTION: The current project will analyze the gut microbiome and metabolites from PD patients and controls, and employ clinically relevant mouse models to determine how metabolites produced by the microbiome from dietary substrates affect motor symptoms. We propose to test whether directly regulating microbial metabolite profiles using "designer" dietary fibers and probiotics offers new avenues for ameliorating PD-like symptoms. During this reporting period 12 new human subjects (75% of targeted enrollment) were successfully recruited at the RUMC site to result in a total of 45 human subjects recruited (75% of targeted enrollment) in the first 3 years which partially meets our proposed goal. We have also made remarkable progress on the animal studies, defining specific diets that impact motor deficits in a mouse model of PD, and initiating mechanism of action studies. We have advanced the objectives of the project either on time, or in some cases, ahead of schedule. The project has, to date, not experienced any major setbacks.

1. **KEYWORDS:** *Parkinson's disease, human subjects, intestinal microbiome, stool specimens, gut-brain axis, intestinal bacteria, dietary fiber, short chain fatty acids*
2. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

<u>Major Task 1: Recruitment and Microbiome Sequencing</u>

Subtask 1- subject recruitment and sample collection.	*12 month target of 17 human subjects with stool and tissue collection successfully recruited.*	*75% completed*
Subtask 2- microbiome sequencing / metagenomics.	*24 month timeline.*	*80% completed*
Subtask 3- SCFA analysis for stool and serum.	*12 month timeline.*	*70% completed*

<u>Major Task 2: Animal colonization and phenotyping</u>

Subtask 1 – colonization of mice with human microbiota	*36 month timeline.*	*75% completed*
Subtask 2 – microbiome profiling.	*36 month timeline.*	*75% completed*
Subtask 2 – motor testing, neuroinflammation status.	*36 month timeline.*	*75% completed*
Subtask 3 – AAV cloning and injection.	*6 month timeline.*	*50% completed*
Subtask 4 – CLARITY analysis and electrophysiology.	*36 month timeline.*	*60% completed*

<u>Major Task 3: Fiber testing and treatment of animals</u>

Subtask 1 – treat PD mice with fibers and motor tests.	*12 month timeline.*	*100% completed*
Subtask 2 – treat PD mice with "optimized" fibers & test	*36 month timeline.*	*100% completed*

- **What was accomplished under these goals?**

Activities accomplished in this quarter include: 1) partially reached our 36 month goal for recruitment, with the target of 49 subjects; 45 subjects were already recruited ; 2) colonization of germ-free WT and ASO mice with human microbiota; 3) SCFA treatment of SPF mice followed by motor testing; 4) feeding of SCFAs to SPF mice and analysis of neuroinflammation; 5) production and treatment of animals with prebiotic fibers, 6) motor testing mice fed prebiotic fibers, and 7) RNAseq of microglia isolated from brains of mice fed prebiotic diets. We are excited to report that acetate feeding to SPF animals showed an effect on motor symptoms. Namely, feeding designer prebiotic diets enriched in 20% butyrate or acetate each improved motor symptoms in mice, whereas the 20% propionate diet did not have this effect, showing specificity for different SCFAs in our mouse model of PD. Further, we show that butyrate reduces activation of microglia in vitro, and thus may affect neuroinflammation in vivo. Finally, we have profiled the transcriptome of microglia from brain regions of mice fed SCFAs, and find very interesting results that diet treatment alters specific immune pathways for microglia activation. There have been no setbacks or failures to achieve a goal, and the project is progressing on the proposed timeline or in some cases such as the microglia studies, ahead of schedule. Finally, we have published 3 major papers in this reporting cycle, both supported by DoD funding.

- **What opportunities for training and professional development has the project provided?**

Research. *Trainees meet weekly with the PI, both separately and together, to discuss their latest results, technical problems, collaborations, reagent needs and so forth.*

Group Meeting & Scientific discussions. *The Mazmanian laboratory holds formal 2 meetings every week, one for research updates from investigators and the other to discuss literature. Each week a group member presents their work. Often, we have PIs, students and fellows from other laboratories join in our weekly meetings. Each trainee presents their work approximately every 4 weeks to the entire group. These lab meetings cover a range of topics, from immunology, neuroscience, behavior to microbiology to animal models of disease. We frequently discuss relevant papers in the field and how they impact the research in our laboratory. Furthermore, each trainee participates in Caltech's vigorous seminar program in which outside scientists come to Caltech to present their research. They also have the opportunity to participate in the weekly "BioLunch", which features two half-hour presentations every week by a student and/or postdoc, thereby providing excellent exposure to ongoing projects in the Biology Division. Further, the 2 postdoctoral fellows will present their work once a year in a campus-wide seminar series called "Micro Mornings", where members of the microbiology community at Caltech discuss their work in front of an audience of peers that include not only biologists, but chemists and engineers as well. The diverse feedback from this worthwhile helps students and fellows craft dynamic research programs. In addition, the students and fellows in the laboratory organize their own weekly journal club, practice talks and brainstorming sessions, often without me.*

Mentoring*. The PI mentors each trainee on science, their careers, ethics, scientific strategy, interpersonal relationships, oral and written communication, graphics, and so forth. I realize that each young scientist has different talent sets, and thus try to help each individual improve all their skills. For example, we discuss appropriate and effective ways to network, how to turn potential competitors into collaborators, how to compete (if necessary) in a collegial way, etc. We also engage in open discussions about alternative career choices in addition to preparation for obtaining and succeeding in an academic career. I view my role as*

a mentor to primarily be a resource for the scholarly, academic and personal advancement of the careers of my trainees.

***Writing**. In general, the PI does not write the research papers from his laboratory, but discusses content, organization and figures as the papers are planned and being written, edits to enhance the personal style of each author, and rewrites key parts if necessary. My goal is to train superb writers. Other laboratory members continually critique each other's manuscripts, grant proposals, research statements, posters, etc.*

***Scientific meetings and conferences.** Trainees attend and present her data at 2 or 3 scientific meetings each year, either locally, nationally or internationally. All trainees have presented their findings from this project at 3 scientific meetings in the past year. This provides not only the opportunity to receive feedback and critique on the project, but to network with researchers*

How were the results disseminated to communities of interest?

The PI has presented work from this project at 5 national and international meetings
The PI is scheduled to present this work at the 2019 World Parkinsons Congress, 2019 Federation of Neurogastroenterology and Motility meeting and 2019 Society for Neuroscience meeting. The PI has presented the data from this study at an additional 5 international meetings focused on either neuroscience or microbiome research.

Reem Abdel-Haq and the PI have authored a review manuscript on the topic of this project that was recently accepted for publication after peer-review in the prestigious Journal of Experimental Medicine

- **What do you plan to do during the next reporting period to accomplish the goals?**

1) In the Year 4 of the Project, Dr. Keshavarzian's team at RUMC will continue vigorous patient and subject recruitment and sample collection (target for Year 4 for RUMC is 11 subjects). So far we have succeeded in hitting 92% of our 3 year enrollment target goal for human subjects recruitment (45/49). 2) Microbiome sequencing and SCFA analysis will be done in batches. 3) Dr. Mazmanian's group will analyze motor symptoms, neuroinflammation and pathophysiology in the "humanized" mouse models following prebiotic treatment. 4) We will evaluate short chain fatty acid (SFCA) levels in the prebiotic treated mice. 5) Dr. Gradinaru's group will image brain tissues from these mice. 6) Drs. Mazmanian and Hamaker will finish the "optimized" prebiotic diets.

3. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

- **What was the impact on the development of the principal discipline(s) of the project?**
- *Rush University Medical Center site and Dr. Keshavarzian's team achieved the targeted new human subject recruitment and enrollment goal (12/16; total 45/49 for 3 years) which is required for the success of the project. The animal studies at Caltech further*

corroborated the preliminary data for a role by SCFAs in motor symptoms in mice. The fecal samples from all subjects collected at Rush are currently being sequenced at UCSD and will be published shortly after bioinformatic analysis.

- **What was the impact on other disciplines?**

Nothing to report

- **What was the impact on technology transfer?**

Nothing to report

- **What was the impact on society beyond science and technology?**

Nothing to report

4. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**

Nothing to report

- **Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to report

Changes that had a significant impact on expenditures

Nothing to report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report

- **Significant changes in use or care of human subjects**

Nothing to report

- **Significant changes in use or care of vertebrate animals.**

Nothing to report

- **Significant changes in use of biohazards and/or select agents**

Nothing to report

5. **PRODUCTS:** *"Nothing to Report."*

- **Publications, conference papers, and presentations**

  Report only the major publication(s) resulting from the work under this award.

  - **Journal publications.**

https://pubmed.ncbi.nlm.nih.gov/32066981/

https://pubmed.ncbi.nlm.nih.gov/32043464/

https://pubmed.ncbi.nlm.nih.gov/32071263/

Reem Abdel-Haq and the PI have authored a review manuscript on the topic of this project that was recently accepted for publication after peer-review in the Journal of Experimental Medicine (PDF provided in previous progress report)

Drs. Mazmanian and Gradinaru published 2 major original research articles in 2020 (PDFs provided in previous progress report)

Dr. Hamaker published a major paper (attached)

- **Books or other non-periodical, one-time publications.**

Nothing to report

- **Other publications, conference papers, and presentations.**

Nothing to report

Website(s) or other Internet site(s)

sarkis.caltech.edu

Technologies or techniques

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

6. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

NAME:	**Sarkis K. Mazmanian, PhD**
PROJECT ROLE:	PI, and Caltech Site PI
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	1.20 Calendar Months
CONTRIBUTION TO PROJECT:	Dr. Mazmanian directs the overall project, as well as the Caltech site as it relates to his laboratory. He meets with the Caltech team weekly, as well as additional ad hoc meetings. He organizes and leads the monthly team call that includes the all groups involved at Caltech, Rush, Perdue, UCSD and U of Wisconsin.
FUNDING SUPPORT (If Applicable):	

NAME:	**John Bostick, PhD**
PROJECT ROLE:	Postdoctoral Fellow, Investigator
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	6.00 Calendar Months
CONTRIBUTION TO PROJECT:	Dr. Bostick leads all studies on the mouse motor testing, neuroinflammatory analysis, and pathophysiology studies.
FUNDING SUPPORT (If Applicable):	

NAME:	**Livia Hecke Morais, PhD**
PROJECT ROLE:	Postdoctoral Fellow, Investigator
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	12.00 Calendar Months
CONTRIBUTION TO PROJECT:	Dr. Morais leads all studies on the humanized mice.

FUNDING SUPPORT (If Applicable):	Dr. Hecke Morais was partially supported by a fellowship from the American Parkinson Disease Association.

NAME:	**Reem Abdel-Haq**
PROJECT ROLE:	Graduate Student, Investigator
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	12.00 Calendar Months
CONTRIBUTION TO PROJECT:	Ms. Abdel-Haq leads the prebiotic feeding studies
FUNDING SUPPORT (If Applicable):	Reem is partially supporting by graduate student fellowship.

NAME:	**Yvette Garcia-Flores**
PROJECT ROLE:	Senior Technician
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	4.62 Calendar Months
CONTRIBUTION TO PROJECT:	Ms. Garcia-Flores supported the team with technical expertise and ordering of reagents
FUNDING SUPPORT (If Applicable):	

NAME:	**Taren Thron**
PROJECT ROLE:	Research Technician
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	4.62 Calendar Months
CONTRIBUTION TO PROJECT:	Ms. Thron assisted the team with animal behavior studies and animal breeding
FUNDING SUPPORT (If Applicable):	

NAME:	**Joseph Boktor**
PROJECT ROLE:	Research Technician
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	7.88 Calendar Months
CONTRIBUTION TO PROJECT:	Mr. Boktor is the lead investigator on the microbiome sequencing component of the project, and is working closely with the UCSD team
FUNDING SUPPORT (If Applicable):	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report

- **What other organizations were involved as partners?**

Nothing to report

7. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:** *N/A*
   - **QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*
8. **APPENDICES:** *N/A*

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PERSPECTIVE
Applied and Environmental Science

New View on Dietary Fiber Selection for Predictable Shifts in Gut Microbiota

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ABSTRACT Dietary fibers can be utilized to shape the human gut microbiota. However, the outcomes from most dietary fibers currently used as prebiotics are a result of competition between microbes with overlapping abilities to utilize these fibers. Thus, divergent fiber responses are observed across individuals harboring distinct microbial communities. Here, we propose that dietary fibers can be classified hierarchically according to their specificity toward gut microbes. Highly specific fibers harbor chemical and physical characteristics that allow them to be utilized by only a narrow group of bacteria within the gut, reducing competition for that substrate. The use of such fibers as prebiotics targeted to specific microbes would result in predictable shifts independent of the background microbial composition.

KEYWORDS dietary fiber, gut microbiota, fiber specificity, fiber response

The human colon harbors a dynamic and complex community of microbes and holds one of the highest cell densities known (1). Microbiota-host interactions not only impact the host digestive tract but are also involved in many immunological and physiological responses that affect distinct body sites and systems (2). Not surprisingly, many gut bacterial species/groups and their produced metabolites have been related to the course, prevention, or treatment of diseases such as diabetes, obesity, hyperoxaluria, ulcerative colitis, and cancer (3). Thus, the manipulation of commensal gut bacteria is a potential strategy in the management of several health conditions (4). Diet has a pivotal role influencing the composition and function of intestinal microbes and, in this sense, has been recently explored as a tool to shape the gut microbiota (4, 5). A typical diet in Western countries supplies the colonic microbes with 13 to 20 g of dietary fiber (DF) daily (6–8) on which commensal bacteria primarily rely to harvest energy and carbon. As carbohydrate polymers and oligomers, DFs are composed of a variety of monosaccharides polymerized through distinct linkage patterns to final molecules of diverse size ranges. They may be further linked to other chemical groups or molecules (e.g., acetyl, methyl, and feruloyl groups) and possess physical variations, such as solubility degree, viscosity, and three-dimensional arrangements. The vast array of simple and complex possible structures means that DFs contain challenging substrates that require sophisticated bacterial machineries to be accessed, degraded, and utilized (9). Microbes possess genetic information to express specific carbohydrate-active enzymes (CAZymes), recognition and binding proteins, and transporters that are required in this process (10). The heterologous expression of this molecular machinery in distinct species results in divergent specialization to ferment discrete fiber structures (9). Conceivably, a dietary fiber structural alignment to specific bacterial abilities would allow selective stimulation of growth and/or activity of microbes associated with health protection and well-being.

CHALLENGES FOR A TARGET-SPECIFIC FIBER APPROACH

Although microbes have specific abilities to utilize distinct DF structures, it seems not so easy to simply give a particular DF to a person to promote a specific gut

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bacterium. For instance, commonly used prebiotic fibers do not present consistent results across individuals. We believe that this is in part because there are easily accessible and simple fiber structures in nature that are utilized by many bacteria (low-specificity fibers). For example, fructooligosaccharides (FOS), a soluble short-chain DF polymer containing mainly $\beta(1\rightarrow2)$-linked fructose that is commonly used in clinical trials to promote shifts in the gut microbiota, was initially associated with the growth of bifidobacteria (11). More recently however, Scott et al. found that of 14 distinct bacterial species tested, including representative members of the *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* phyla, all were able to grow well on FOS using *in vitro* single-culture experiments (12). An *in vivo* study using 16S rRNA gene sequencing analysis further showed that many other bacteria (102 taxa) were stimulated or inhibited to some extent by FOS supplementation in mice (13).

The coexistence of many different microbes able to utilize the same DF, accompanied by high cell densities relative to the available nutrient resources, results in competitive pressures within the gut that dictate different fiber response outcomes (14). In that way, the bacterial groups stimulated by a given low-specificity DF will differ when community pressures are different, i.e., in distinct microbial communities. Venkataraman et al. evaluated butyrate production during *in vitro* fecal fermentation of resistant starch, another DF that many bacteria have the ability to utilize (15). The results presented show varied responses in samples from different subjects, presumably due to distinct initial characteristics of their gut microbiota. Recently, Johnson et al. conducted a human study, without diet interventions, to investigate how dietary patterns relate to microbial shifts based on daily fecal sampling and dietary records (16). Although they found that diet significantly alters the gut microbiota, distinct personalized bacterial responses were observed among individuals consuming the same groups of food. This is likely due to the DF response being dependent on the composition of the microbial community. In a recent study with germ-free mice fed a diet containing arabinoxylan and colonized with an artificial community, including the arabinoxylan degraders *Bacteroides ovatus* and *Bacteroides cellulosilyticus*, *B. ovatus* increased only when *B. cellulosilyticus* was absent, showing that fiber response is closely related to microbial community layout (17). Chen et al. showed that fecal ferments with an initial dominance of *Prevotella* spp. versus *Bacteroides* spp. respond differently to DFs regarding both bacterial shifts and metabolites produced during *in vitro* fermentation (18). Notably, classifying the human microbiota only by enterotype (*Prevotella* spp. versus *Bacteroides* spp.) is a generalist approach (19), and it is probable that distinct fiber responses happen within individuals classified in the same enterotype due to divergences in microbial communities. Data from the Human Microbiome Project (20) show that although individuals have up to several hundred species of microbes within their gut, thousands or more different species inhabit the gut of human populations collectively, which confirms a high degree of variation in microbiota composition among individuals. From a functional point of view, such high variability would also infer a range of divergent (and perhaps unpredictable) responses when a low-specificity fiber is given to different individuals (18, 20). Thus, the use of these fibers to sustain the growth of targeted bacteria in a predictable way in every individual hardly seems an achievable goal.

On the other hand, fibers with higher specificity (i.e., accessible and fermentable by a limited number of bacteria) could promote specific taxa independent of the competitive pressures of the environment for nutrient acquisition. As an example, Shepherd et al. colonized three groups of mice harboring distinct microbiota communities with a rare *Bacteroides ovatus* strain isolated specifically for its ability to utilize both inulin and the polysaccharide porphyran (21). Because no other bacteria in mouse intestinal communities could utilize porphyran, its administration led to a targeted, predictable, and dose-dependent increase in this rare *B. ovatus* population. Moreover, different *B. ovatus* growth responses were observed among the three mice microbiotas when inulin was used as the only energy source; however, when porphyran was utilized, the growth rate was consistent independent of the background microbiota (21). We have

also shown that specific and unusual dietary fibers can be utilized to target the bacterial growth of species of biological significance that naturally occur in the gut. Using a fungal insoluble β-(1-3)-linked glucan, a genus of butyrate-producing bacteria (*Anaerostipes*) was specifically stimulated in an *in vitro* human fecal fermentation, increasing abundance in 24 h from <0.5% of the total bacteria in the initial inoculum to approximately 24% (22). It seems that this fiber is highly specific for these bacteria, with not many other microbes in the gut having the ability to compete and utilize these β-glucans, although an ecological effect cannot be dismissed and is under investigation. Also, entrapment of starch into alginate microspheres was shown to reduce starch utilization by *Bacteroidetes* species and specifically promoted butyrogenic *Firmicutes* (23). While the starch utilization system (Sus) in *Bacteroidetes* requires physical attachment to degrade and utilize starch (24), *Firmicutes* employ cellulosome-like appendages or secrete starch-degrading enzymes with no need for direct physical attachment to the fiber, taking advantage of the inaccessible alginate-entrapped starch for growth.

HIERARCHICAL DIETARY FIBER MODEL FOR MICROBIAL SPECIFICITY

We propose that dietary fibers can be generally classified hierarchically according to their specificity to gut microbes (Fig. 1A). On the top of the hierarchy are low-specificity fibers that are easily accessible and utilized by many colonic microbes, resulting in competitive pressures to utilize these nutrients (Fig. 1A) and variance in the response related to an individual's gut microbiota community structure (Fig. 1B). One could classify FOS and inulin as low-specificity DFs because many bacterial taxa are able to access and degrade them. The fermentation response to these DFs largely rely on microbes' ability to compete among each other to utilize them, with competitive pressures varying among individuals as much as microbial community composition differs. Thus, the use of low-specificity dietary fibers would generate divergent fiber responses across individuals. On the other hand, at the bottom of the hierarchy are high-specificity fibers, such as the above-mentioned insoluble β-glucans, that possess structural features that only few bacteria can access, degrade, and utilize efficiently. These include DFs with both complex chemical (sugar compositions and linkage combinations) and physical (e.g., insoluble matrix fibers) structures. Due to the more specific alignment of these DF structures and lower number of utilizing bacteria, competition for these highly specific fibers is reduced. With a limited number of microbes able to access and degrade them, high-specificity DFs promote a more targeted action toward their utilizers (Fig. 1A). Distinct intermediate levels of fiber specificity may take place according to physicochemical structures that would confer the fibers with a higher or lower degree of specificity. We believe that the reduced competitiveness for high-specificity fibers allows a more predictable and similar fiber response in a population, even in individuals harboring distinctly different microbial communities but that contain the target bacteria (Fig. 1B). Importantly, the targeted bacteria to be promoted by highly specific fibers should be either naturally present in one's microbiota, as shown by Cantu-Jungles et al. (22), or supplemented as a probiotic with the addition of the highly specific fiber as shown by Shepherd et al. (21). We acknowledge that a given target bacterium may not be prevalent in a population, but still, the high-specificity fiber approach would be valid as a prebiotic if the bacterium is present or synbiotic if absent.

Many other fermentable DFs, such as arabinogalactans, mannans, xylans, arabinoxylans, xyloglucans, pectins, resistant starch, soluble glucans, and arabinans, exist in nature (25). Also, distinct fiber characteristics (as discussed in Dietary Fiber Characteristics That May Affect Specificity) within the same class of dietary fiber would confer a higher or lower specificity toward a targeted gut microbe. A comprehensive classification of all distinct DFs regarding specificity to gut bacteria is yet a matter of investigation but could conceivably result in a compilation of a library of DF structures to support a range of beneficial gut bacteria (9). This would necessitate a further understanding of key bacterial species that are important to gut health and then a determination of the DF types and structures that support them. Moreover, cross-feeding

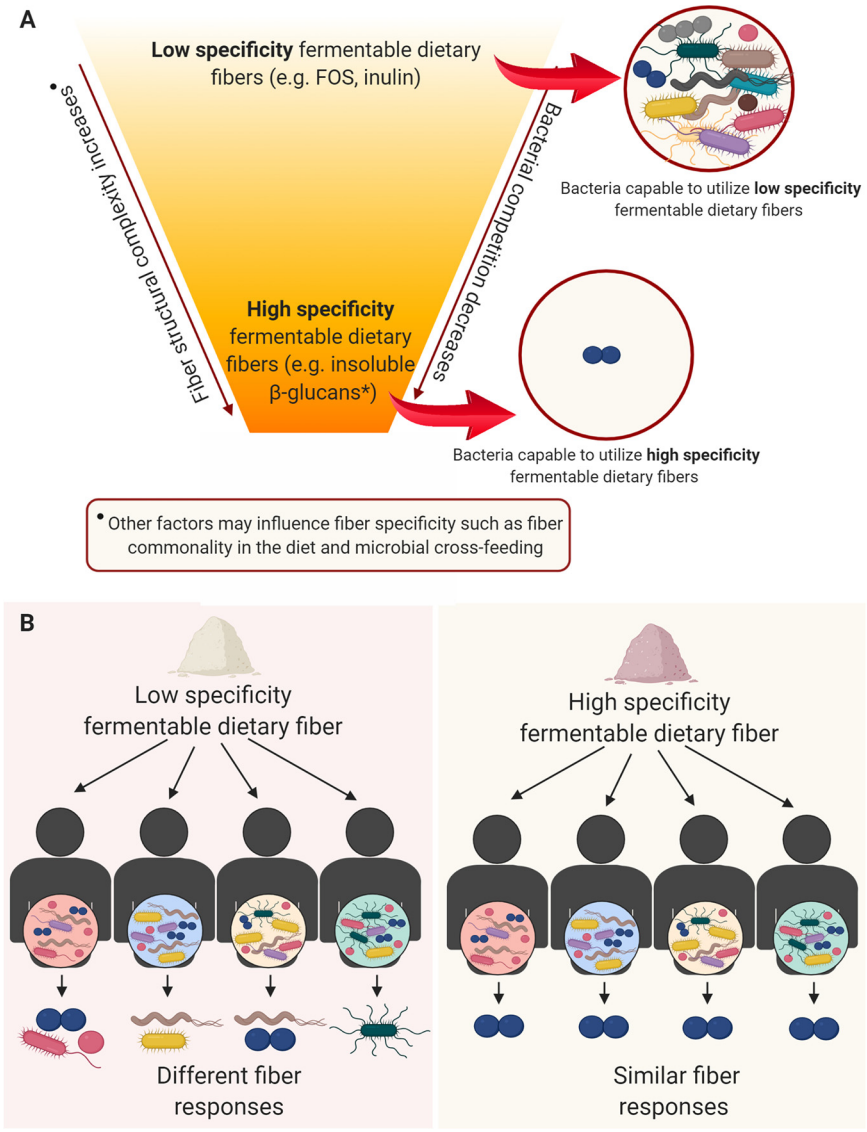

FIG 1 (A) A hierarchical view of dietary fiber specificity toward gut microbes. *, Cantu-Jungles et al. (22). (B) Fiber responses among individuals using low- versus high-specificity dietary fibers.

occurs within gut commensals and further increases the number of species that benefit from the presence of a given DF (26). Yet, there is also evidence that bacteria are not strictly assigned as cross-feeders, and when other carbohydrate sources are available, they can directly utilize them (27). Nonetheless, discrete fiber characteristics, including chemical and physical structures, are important features to DF specificity and could be selected or manipulated to increase a DF's specificity to a particular bacterium or bacterial group.

DIETARY FIBER CHARACTERISTICS THAT MAY AFFECT SPECIFICITY

Physicochemical complexity. The molecular machinery necessary to ferment a fiber is structure specific; hence, DF chemical and physical structures largely influence which bacteria will access and ferment them (9, 28, 29). More-complex DF chemical structures (e.g., those containing a variety of sugars, linkage types, and branching patterns) require many bacterial enzymes to act in synergy for their complete saccharification, and, from a rational point of view, there is a tendency that the more complex the DF, the fewer the bacteria in the gut capable of fermenting it. For instance, while many *Bacteroides* species can grow on xylose and glucose, only a limited number of

taxa harbor the genetic machinery to grow in xyloglucans (30, 31). Recently, Ndeh et al. explored the ability of 29 *Bacteroidetes* strains to grow on type II rhamnogalacturonan, a pectic polymer containing 21 distinct glycosidic linkages (32). Less than one-third of these organisms grew on the glycan. Scott et al. have shown that polymerization degree also influences fiber specificity. In single bacterial cultures, all 15 evaluated taxa (10 representative *Firmicutes*, 3 *Bifidobacterium* spp., and 2 *Bacteroides* spp.) could grow on short-chain FOS, but only five of them grew on a long-chain inulin (12). Overall, these data indicate that more-complex fiber structures are likely to be more selective to specific bacteria than are simple DF polymers.

While there are reports of human supplement studies of specific responses to more simple dietary fibers, upon close inspection of the results, one sees varied responses ranging from increases to decreases in both metabolites and target bacteria. For instance, galactooligosaccharides were shown to specifically increase *Bifidobacterium* spp.; however, only 11 out of the 18 subjects tested had actual increases in *Bifidobacterium* spp. (33). Also, Vandeputte et al. (34) reported specific responses to inulin-based fructans, but there was high variability around the mean for *Bifidobacterium* spp., suggesting responders and nonresponders. Baxter et al. (35) showed that in 43 individuals supplemented with resistant starch, only 22 individuals responded with a butyrate increase, while 21 individuals responded with a reduction or no changes in fecal butyrate concentration. Bacterial shifts, including those of *Ruminococcus bromii*, a known starch degrader, also showed high variability among subjects.

Another important point regarding fiber specificity to bacteria involves physical properties, such as insolubility degree, that reduce the accessibility of DFs by microbes and provide an additional challenge for attachment and enzymatic degradation. Leitch et al. demonstrated that specialized groups from the *Firmicutes* phyla, such as *Clostridium* clusters *IV* and *XIVa,* are more associated with insoluble particles in human feces than are *Bacteroidetes* (36). Differences in bacterial motility may be particularly important in colonizing this kind of substrate. For instance, *Roseburia inulinivorans*, a bacterium from the *Clostridium* cluster *XIVa*, had genes related to flagellar synthesis that are upregulated during growth on starch (insoluble fiber) but not on inulin (soluble fiber) (37). Moreover, bacteria possessing cellulosome-like appendages, which allow bacteria to access insoluble substrate matrices, also have an advantage in the utilization of these polymers (38). Thus, DF specificity to bacteria with these kinds of apparatuses could be increased by the utilization of its insoluble forms. Physical accessibility can also be manipulated to increase specificity to a target group of microbes. We have recently shown that a solubilized corn arabinoxylan that was cross-linked to form soluble matrices shifted growth toward butyrogenic *Clostridia* bacteria (39). Also, as mentioned above, raw starch entrapped in porous alginate microspheres was shown to promote butyrogenic *Firmicutes* in mice, with a reduction in *Bacteroidetes* species that must physically attach to normal resistant starch by the Sus assembly to utilize it (23). *Firmicutes* harbor distinct starch utilization strategies that do not require physical attachment. There is some evidence that other physical characteristics are relevant during DF fermentation as well. In mice, divergent bacterial populations in the cecum were promoted by the same diet in different physical forms (powdered versus pelleted) (40). Also, increases in viscosity were related to the growth of total anaerobes and *Clostridium* spp. in a gastrointestinal simulator inoculated with fecal microbiota, and the decrease in viscosity was related to *Enterococcus* sp. growth (41). Recently, Tuncil et al. showed that in *in vitro* fecal ferments, larger wheat bran particles selected toward a more butyrogenic microbiota, while smaller particles were associated with a more propiogenic microbiota (42). Examples of how fiber structural complexity is addressed by bacteria are illustrated in Fig. 2.

Other factors that may affect fiber specificity. Besides dietary fiber physicostructural complexity, we believe that some other factors may influence the degree of fiber specificity, such as DF commonality in diets and utilization through bacterial cross-feeding.

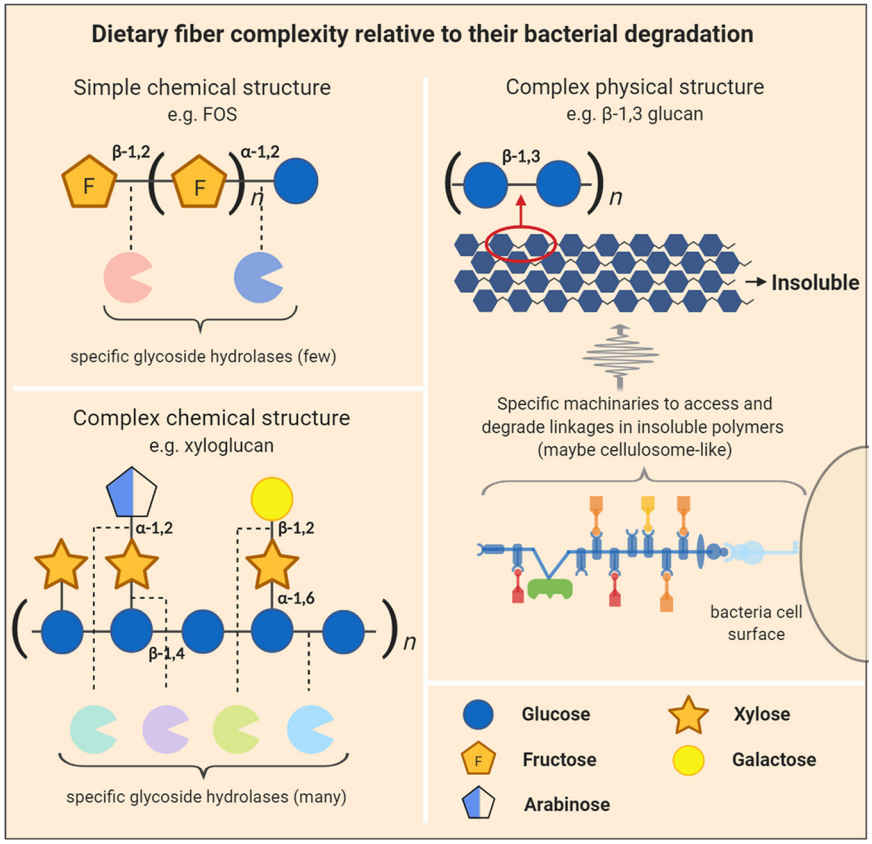

FIG 2 Dietary fiber complexity relative to their bacterial degradation. Dietary fibers with simple chemical structures, such as fructooligosaccharides (FOS), require few bacterial glycoside hydrolases to degrade them, whereas more-complex molecules, such as xyloglucans, which contain a range of sugar and linkage types, require that bacteria have more glycoside hydrolases for their complete degradation. Complex physical structures, such as those found in insoluble dietary fibers (e.g., β-1,3 glucan [22]), also require that bacteria have specific and perhaps more complex machinery to access these insoluble substrates (maybe cellulosome-like appendages).

From an evolutionary point of view, it is plausible to think that fewer bacteria in the human gut are equipped to digest DFs that are rarely consumed in the diet. Bacterial genes not often utilized confer a fitness cost to the bacterium, which might drive to an adaptive process to get rid of these superfluous genes (43), such as those related to the digestion of DFs uncommonly consumed in the human diet. In fact, observations from a synthetic microbial community from the human gut show that the ability to grow in rarely consumed DFs, such as laminarin (from algae) and lichenin (from lichens), is restricted to few bacteria (44). As previously discussed, in our research group, a linear insoluble β-1,3 glucan resulted in the growth of specific bacteria in a human fecal gut community (22). Besides the complex physical structure (i.e., insoluble form) of the glucan, β-1,3 linkages between glucose units are mainly found in fungi, oomycetes, and lichens (45) and are not often consumed in large amounts in the human diet, therefore increasing its specificity to certain microbes. Accordingly, common DFs are likely to have multiple utilizing gut bacteria, while uncommon DFs would be utilized by few bacteria and therefore present more similar fiber responses among individuals who have the target bacteria.

Dietary fibers that support a limited number of bacteria would likely not be involved in cross-feeding, which involves degradation by a keystone species to release DF fragments or simple metabolic products that are used by other gut bacteria. A good example is resistant starch, which is degraded and utilized by a group of bacteria through cross-feeding, thus leading to less specificity (35). Thus, fibers that are more common in diets and are utilized through cross-feeding tend to have lower specificity,

while fermentable fibers that are uncommon in the diet and those that do not result in cross-feeding tend to have higher specificity.

Overall, physicochemical fiber characteristics and these other factors combine to give DFs properties of low to high specificity regarding their utilization by gut bacteria.

CONCLUSIONS

We believe that a niche differentiation of taxa with unique abilities to ferment highly specific DF structures naturally occurs in the gut. The design and selection of high-specificity DFs as the substrates for the modulation of the gut microbiota would prevent resource competition, resulting in more targeted and predictable shifts in specific taxa independent of the overall microbiota composition.

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