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Gut check: microbiome patent update

By Mark J. FitzGerald and David S. Resnick

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Trends in issuing patents:
Some faithful Gut Check readers have noticed the recent hiatus in new issues. In that time, the editors have been actively working to protect their clients' interests in microbiome-related and non-related technologies, as well as attending and presenting at microbiome conferences in the U.S. and abroad. The time away from publishing Gut Check has provided confirmation that the field is fairly rapidly moving toward species- or strain-level identification of microbes in patent claims. A surprise, however, after hearing time and again from researchers that similarity to 16S rRNA is imprecise and waning in utility, has been seeing that patent claims reciting 16S rRNA similarities continue to issue. Some of this is due to the ongoing prosecution of new claims based on priority applications first filed several years ago. However, we'll look at the trend and consider its implications in the following.

A look at issuing patents: 16S rRNA insists "But I'm not dead yet."

An ongoing issue in microbiome-related IP is how to describe the subject microorganisms. Under 35 USC §112, claimed subject matter must be definite—that is, the boundaries of what is claimed must be apparent to one of ordinary skill in the art—and the patent specification has to describe the claimed subject matter in a manner that demonstrates that the Applicant had possession of the full scope of that which is claimed. Where the desired function performed by any given microorganism can likely be performed by a number of other microorganisms, it becomes important not to define the microbes too narrowly, but if they are defined too broadly, the claims may be subject to challenge for encompassing more than what the Applicant reasonably possessed. The degree of similarity to the 16S rRNA of a reference microbe is a device that has been used in patent claims to gain breadth around a given microbe, on the theory that closely related species will share a high degree of sequence identity at the 16S rRNA level. However, two microbes can share identical 16S rRNA sequences yet have differing functional characteristics. In such instances, it can help to provide description of microbes in more elaborate detail, whether at the specific strain level, the genomic level, the transcriptomic level or some combination thereof in a patent's specification and claims.

It is stressed here that 16S rRNA sequence similarity is in no way inherently improper or insufficient in any given case under U.S. patent law. Any given claim will depend upon the description of microbes that have the desired functional and structural characteristics—the greater the number of different microbes described that have the desired characteristics and the greater the detail provided regarding them and what sets them apart from microbes that do not have the desired function, generally the broader the claim language permissible. The following examples include recently issued claims that recite percent identity to 16S reference sequences alone, generally at the 95%, 97% or greater level of identity; percent identity to 16S reference sequence together with a function; percent identity to a 16S reference sequence together with a function linked to another structure; and alternatives that do not rely upon 16S at all.

Claims defining bacteria by 16S sequence only

U.S. Patent 10,052,353

- Titled: Human-derived bacteria that induce proliferation or accumulation of regulatory T cells
- Inventors: Honda, et al.
- Assignees: The University of Tokyo (Tokyo, JP)
- School Corporation, Azabu Veterinary Medicine Educational Institute (Sagamihara-shi, Kanagawa, JP)
- Issued: August 21, 2018

Claim of interest:

- 1. A pharmaceutical composition comprising a purified bacterial mixture comprising **two or more bacterial strains comprising 16S rDNA sequences of at least 95% homology to SEQ ID NO**:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41 **or** SEQ ID NO:42; and one or more enteric polymers.

Microbes that promote proliferation or accumulation of Tregs are identified in the specification by genus, species and strain, and by OTU sequences of ~320 nucleotides of 16S rDNA. The specification also refers to "homology with DNA of one or more of the following" microbes specified by genus, species and/or strain, but genomic sequences are not provided.

The noted claim defines the subject consortium of any two or more bacterial strains having at least 95% *homology* to the specified SEQ ID Nos, which correspond to certain partial 16S sequences.

U.S. Patent 10,058,578

- Titled: Compositions and methods comprising a defined microbiome and methods of use thereof
- Inventors: Honda, et al.
- Assignees: The University of Tokyo (Tokyo, JP)
- School Corporation, Azabu Veterinary Medicine Educational Institution (Sagamihara-shi, Kanagawa, JP)
- Issued: August 28, 2018

Claim of interest:

- 1. A pharmaceutical composition comprising a purified bacterial mixture consisting of **bacteria comprising 16S rDNA sequences of at least 95% homology to SEQ ID NO**:22, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:41 **and** SEQ ID NO:42; wherein the bacterial mixture induces the proliferation and/or accumulation of regulatory T cells, and one or more enteric polymers.

This patent has the same disclosure as the '353 patent above. The noted claim defines the subject consortium by at least 95% *homology* to the partial 16S sequences listed. Note that the claim requires a combination of bacteria having each of the sequences listed.

This claim refers to the function of inducing proliferation and/or accumulation of Tregs; it's listed here under "16S only" because the recited function is an effect on the host, rather than, for example, a specific process carried out by the bacteria—this is admittedly a somewhat arbitrary distinction, but reference to specific processes the bacteria perform, e.g., a specific enzymatic conversion, may carry more descriptive weight.

U.S. Patent 10,064,900

- Titled: Methods of populating a gastrointestinal tract
- Inventors: Von Maltzahn, et al.
- Assignees: Seres Therapeutics, Inc. (Cambridge, MA)
- Issued: September 4, 2018

Claim of interest:

- 1. A method of treating or reducing the severity of at least one sign or symptom of a gastrointestinal disease in a human subject, the method comprising administering to the human subject an effective amount of a composition consisting essentially of a purified population of germinable bacterial spores, **wherein the population of germinable bacterial spores includes at least a first Operational Taxonomic Unit (OTU) and a second OTU, the first OTU comprising a first 16S rDNA comprising a first sequence having at least 97% identity to SEQ ID NO: 381 and the second OTU comprising a second 16S rDNA comprising a second sequence having at least 97% identity to SEQ ID NO: 556**, wherein the effective amount treats or reduces the severity of the at least one sign or symptom of the gastrointestinal disease in the human subject.

This claim describes microbes in terms of percent identity to 16S-based OTUs. Language in the specification defines OTUs by whole genome sequence, multilocus sequence tags (MLSTs), specific genes or sets of genes and 16S sequence or a portion thereof, but only 16S is referred to in the claims as filed or as issued.

U.S. Patent 10,076,546

- Titled: Network-based microbial compositions and methods
- Inventors: Henn, et al.
- Assignees: Seres Therapeutics, Inc. (Cambridge, MA)
- Issued: September 18, 2018

Claim of interest:

- 1. A method of treating or preventing a dysbiosis in a human subject, comprising administering to the human subject a formulation in an amount effective to treat or prevent the dysbiosis or to reduce the severity of at least one symptom of the dysbiosis in the human subject to whom the formulation is administered, **the formulation consisting essentially of a purified population of bacterial spores** in an amount effective to populate the human subject's gastrointestinal tract under conditions such that at least one species of bacteria not detectably present in the purified population of bacterial spores or in the human subject's gastrointestinal tract prior to administration is augmented, wherein the formulation is provided as an oral finished pharmaceutical dosage form including at least one pharmaceutically acceptable carrier, the dosage form comprising at least about $1x10^4$ colony forming units of the bacterial spores per dose of the formulation, wherein the **bacterial spores comprise at least two bacterial species**, each of the at least two bacterial species **comprising a different 16S rDNA sequence at least 97% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO**: 1670, SEQ ID NO: 1591 and SEQ ID NO: 607.

This patent includes substantially the same definition of OTU as in the '900 patent above. The specification and claims as filed also designated therapeutic compositions comprising bacteria of specified clades ultimately defined by genus, species and 16S sequence identity. The claims as issued define the bacterial species only by % identity to specified 16S sequences.

U.S. Patent 9,914,979

- Titled: Method and kit for characterizing microorganisms
- Inventors: Fry, et al.
- Assignees: FRY LABORATORIES, LLC (Scottsdale, AZ)
- Issued: March 13, 2018

Claim of interest:

- 1. A method of identifying a species of one or more microorganisms in a human clinical sample, the method comprising the steps of:
    a) extracting nucleic acids from the human clinical sample, the human clinical sample comprising one or more microorganisms;
    b) preparing from a 16S rRNA gene only first and second target sequences from the extracted nucleic acids from the human clinical sample by
        i. contacting the extracted nucleic acids with a first set of primers comprising SEQ ID NOs:33 and 35-50 for selective amplification of the first target sequence comprising hypervariable region V1 and hypervariable region V2 of a 16S rRNA gene, wherein the first target sequence does not include hypervariable region V3 of the 16S rRNA gene;
        ii. contacting the extracted nucleic acids with a second set of primers for selective amplification of the second target sequence comprising hypervariable region V5 and hypervariable region V4 of the 16S rRNA gene, wherein the second target sequence does not include hypervariable region V3 of the 16S rRNA gene; and
        iii. amplifying the first and second target sequences;

c) sequencing from 5' to 3' V1 and V2 of the first target sequence and sequencing from 5' to 3' V5 and V4 of the second target sequence to obtain sequence reads of the first and second target sequences;

d) identifying the species of the one or more microorganisms in the human clinical sample based on the sequence reads of the first and second target sequences by

   i. comparing each of the sequence reads having greater than or equal to a predetermined length to data in a library; and

   ii. determining sequence reads that correspond to data in the library based on predetermined criteria, wherein the sequence reads identify the species of the one or more microorganisms.

This patent relates to diagnostic methods for identifying, at the species level, microorganisms in a human clinical sample. The specification and claims focus on 16S rRNA gene sub-sequences and their similarity to sequences in a database. The claimed method assigns a species for clinical diagnostic purposes without requiring full 16S sequence. It is noted that clinical diagnosis of infection does not generally require the functional precision for strain identification needed for therapy based on administration of microbes.

Claims defining bacteria by 16S sequence plus genus and species name

U.S. Patent 10,086,021

- Titled: Compositions comprising bacterial strains
- Inventors: Jeffery, et al.
- Assignees: 4D Pharma PLC (GB)
- Issued: October 2, 2018

Claim of interest:

- 1. A method of increasing microbiotic diversity or maintaining microbiotic stability in a gastrointestinal tract of a subject in need thereof, comprising administering to the subject a pharmaceutical composition that comprises an effective amount of **a bacteria strain of the species *Blautia hydrogenotrophica*** sufficient for increasing microbiotic diversity or maintaining microbiotic stability in the gastrointestinal tract of the subject relative to an amount of microbiotic diversity or microbiotic stability prior to the administering, **wherein the bacteria strain comprises a 16s rRNA gene sequence of SEQ ID NO:5**, and wherein the administering results in an increase in an amount of a bacteria from at least one genus selected from Clostridium, Bifidobacterium and Prevotella in the gastrointestinal tract of the subject relative to an amount of the respective bacteria prior to the administering.

This claim defines the subject strain by genus, species and exact identity to the reference 16S rRNA gene sequence.

U.S. Patent 9,987,311

- Titled: Compositions comprising bacterial strains
- Inventors: Mulder, et al.
- Assignee: 4D Pharma Research Limited (Aberdeen, GB)
- Issued: June 5, 2018

Claim of interest:

- 1. A pharmaceutical composition that reduces inflammation associated with Th17 differentiation in a subject in need thereof, that comprises a therapeutically effective amount of a **single bacteria strain of the species *Erysipelatoclostridium ramosum***; and a pharmaceutically acceptable excipient, diluent or carrier; wherein the therapeutically effective amount comprises from about $1x10^3$ to about $1x10^{11}$ CFU of the bacteria strain; wherein the bacterial strain is lyophilized; and wherein the **bacteria strain comprises a polynucleotide sequence of a 16s rRNA gene that has at least 95% sequence identity to the polynucleotide sequence of SEQ ID NO:3**, as determined by a Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12, a gap extension penalty of 2 and a Blocks Substitution Matrix (BLOSUM) of 62.

This patent provides 16S sequence and whole genomic sequence for three strains. The specification provides support for combinations of percent 16S sequence identity and percent genomic sequence identity generally or in specified areas near the 16S genes.

The noted claim defines the subject microbe by genus and species name and 95% 16S sequence identity to one reference sequence.

Claims defining bacteria by 16S sequence plus function

U.S. Patent 9,974,815

- Titled: Compositions comprising bacterial strains
- Inventors: Mulder, et al.
- Assignee: 4D Pharma Research Limited (Aberdeen, GB)
- Issued: May 22, 2018

Claim of interest:

- 1. A method of treatment of cancer in a subject in need thereof, comprising: administering to the subject a therapeutically effective amount of a pharmaceutical composition that comprises: a **bacteria strain with a 16s rRNA gene sequence with at least 99.5% sequence identity to the polynucleotide of SEQ ID NO:2**, wherein the sequence identity is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, and a BLOSUM matrix of 62; wherein **said bacteria strain has a carbohydrate fermentation profile that comprises**: (i) a **positive fermentation of at least one of: L-arabinose and D-xylose**; and (ii) **an intermediate fermentation of Methyl-.alpha.D-glycopyranoside**; wherein said bacteria strain is present in an amount sufficient for treatment of cancer in the subject, and wherein the cancer is a solid tumor cancer.

This patent disclosure includes a wealth of descriptive features for the subject microbes: 16S sequence, whole genome sequence, sequence of a plasmid, carbohydrate fermentation profiles, enzyme profiles and some specific metabolites. The noted claim uses high (at least 99.5%) sequence identity and fermentation profile characteristics to define the active microbe.

U.S. Patent 9,993,507

- Titled: Methods of improving milk production and compositional characteristics
- Inventors: Embree, et al.

- Assignee: Ascus Biosciences, Inc. (San Diego, CA)
- Issued: June 12, 2018

Claim of interest:

- 1. A method for increasing milk production or improving milk compositional characteristics in a ruminant, the method comprising: a) orally administering to a ruminant an effective amount of a ruminant supplement comprising: i. a purified microbial population that comprises **a bacteria with a 16S nucleic acid sequence and/or a fungi with an ITS nucleic acid sequence, which is selected from the group consisting of: SEQ ID NOs: 1-60 and 2045-2107**; and ii. a carrier suitable for ruminant administration, **wherein at least one of the bacteria or fungi are capable of converting a carbon source into a volatile fatty acid** selected from the group consisting of: acetate, butyrate, propionate or combinations thereof; and **wherein at least one of the bacteria or fungi are capable of degrading a soluble or insoluble carbon source**; and wherein the ruminant administered the effective amount of the ruminant supplement exhibits an increase in milk production or improved milk compositional characteristics, as compared to a ruminant not administered the ruminant supplement.

This patent describes 122 bacterial and fungal species with the desired characteristic of influencing milk production or compositional characteristics in a ruminant; these have deposit information and 16S sequence provided. Nearly 2000 additional microbes are named.

The claims as originally filed defined the subject microbes *via* 97% sequence identity to the 122 species of bacteria and fungi 16S and ITS sequences, respectively, combined with the functional characteristics of volatile fatty acid production and carbon source degradation noted in the issued claims. The claims were amended to recite *identity* to the specified 16S and ITS sequences before issue. Thus, the claims define the subject microbes by exact identity in 16S or ITS sequence, coupled with function.

Claims using alternative microbe definition schemes

U.S. Patent 9,968,643

- Titled: Selection and use of lactic acid bacteria preventing bone loss in mammals
- Inventors: Connolly, et al.
- Assignees: BIOGAIA AB (Stockholm, SE), MICHIGAN STATE UNIVERSITY (East Lansing, MI)
- Issued: May 15, 2018

Claim of interest:

- 1. A method for the treatment or prevention of bone loss, comprising administering to an individual in need thereof a lactic acid bacterial strain comprising a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of the genome of *L. reuteri* JCM 1112 (SEQ ID NO: 1), and harboring an identical nucleotide relative to the genome of *L. reuteri* JCM 1112 (SEQ ID NO: 1) in at least one of the following four positions: C in base pair 271 391, G in base pair 453 538, G in base pair 529 228 and C in base pair 599 338, *wherein the lactic acid bacterial strain is L. reuteri ATCC PTA 6475.*

This claim is interesting because it at least initially defines the subject bacterial strain on the basis of 95% identity to the whole genomic sequence of the reference *L. reuteri* strain JCM 1112 (SEQ ID

NO: 1). This would encompass a potentially large number of bacteria that may or may not have the desired functional characteristics. This potential problem is addressed by the requirement that subject bacteria have specific sequence/nucleotides at specified locations. In this instance, two strains of *L. reuteri* were examined, ATCC PTA 6475 and ATCC PTA 4659. According to the specification, whole genome sequencing showed a small set of four SNPs constituted the differences between the genomes of the two strains. The key is that ATCC PTA 1675 provided benefit in preventing bone loss, while the other strain, ATCC PTA 4659 ***did not***. Thus, the difference at these four SNPs is likely related to their function in preventing bone loss, and provides the basis for the claim language defining the strain.

The Applicants amended the claims relatively late in prosecution to require that the strain is ACTT PTA 1675 to address an art rejection. There was no §112 written description rejection. That is, the requirement for the specific strain recited at the end of the claim (italics above) was not added to address a §112 disclosure issue. This approach of defining on the basis of similarity at the whole genome sequence level, combined with reference to specific sequences at specified parts of the genome seems an elegant way to define the subject bacteria while providing some reasonable breadth. Options where fact patterns differ would seem to include, for example, percent identity to whole genome sequence combined with call-outs requiring specific sequences for key pathway enzymes, or with call-outs for specific enzyme sequences in RNA for any subject strain.

U.S. Patent 9,925,222

- Titled: Gut barrier dysfunction treatment and prevention
- Inventors: Mani, et al.
- Assignees: Albert Einstein College of Medicine, Inc. (Bronx, NY)
- Issued: March 27, 2018

Claim of interest:

- 1. A method of treating or preventing gut barrier dysfunction, an illness associated with gut barrier dysfunction, toxic or inflammatory injury to intestines or leaky intestinal syndrome in a subject comprising administering to the subject a therapeutic effective amount of **a bacterium**, **wherein the bacterium is *Clostridium sporogenes* comprising a tryptophanase operon, wherein in the presence of tryptophan, the tryptophanase operon is induced and the bacterium produces indole-3-propionic acid or indoleacetic acid from tryptophan**, wherein pregnane X receptor (PXR) is activated in gut apical enterocytes, thereby decreasing intestinal permeability and inflammation.

This claim defines the subject microbe by genus and species names, combined with the presence of a specified operon and the functional requirement that the operon performs the specified task under the specified conditions. This claim nicely marries structure to function in a manner that seems to satisfy the definition and disclosure requirements of §112. Structure alone, i.e., the presence of the tryptophanase operon, does not guarantee the expression of the operon's genes. Similarly, function alone is not necessarily sufficient given recent emphasis on structure in §112 jurisprudence. Combining both in the manner set out in this claim would appear to be one way to solve the problem in elegant fashion.

Conclusions:

Claims defining bacteria on the basis of 16S sequence similarity continue to issue from the USPTO. This likely reflects the facts that 1) while imperfect, 16S remains a reasonable way to identify

bacteria likely to have similar function, and 2) patents issuing today are often the result of applications filed several years before. For applicants filing today, it is wise to consider 16S as but one of a range of ways to define bacteria, describing alternatives where applicable. Genomic sequence (structural) and transcriptomic (structural and functional) descriptions, often in combination with reference to specific genes or pathways and what they do, are gaining in importance.

For more information on the content of this alert, please contact your regular Nixon Peabody attorney or:

- Mark J. FitzGerald at **mfitzgerald@nixonpeabody.com** or 617-345-1058
- David S. Resnick at **dresnick@nixonpeabody.com** or 617-345-6057