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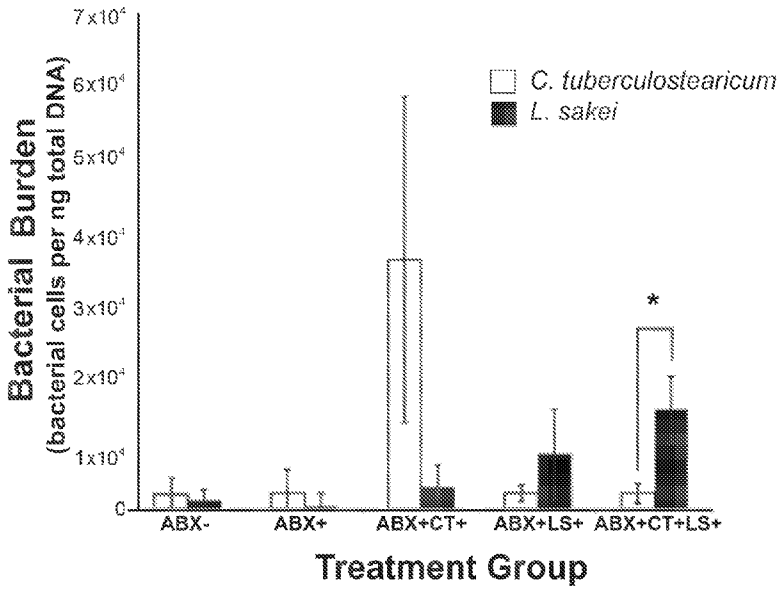

(57) Abstract: Provided herein are compositions for improving sinus microbiota and treating sinusits. Further provided are methods of detecting imbalance in the sinus microbiota that can be indicative of sinusitis, and methods of determining whether an individual has or is at risk of developing sinusitis, e.g., chronic sinusitis.

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SINUSITIS DIAGNOSTICS AND TREATMENTS

BACKGROUND OF THE INVENTION

[0001] Sinusitis (rhinosinusitis) is one of the most common problems presented to the primary care practitioner in the ambulatory setting, affecting more than 15% of the US population annually, resulting in over $5.8 billion in direct health care expenditures (*1*). Typically classified by duration of symptoms, sinusitis may be acute (less than 4 weeks in duration), sub-acute (4-12 weeks) or chronic (more than 12 weeks, with or without acute exacerbations).

[0002] Chronic Rhinosinusitis (CRS), represents a large portion of sinusitis cases, affecting more than 30 million Americans (*2*), resulting in an annual economic health care burden in excess of $2.4 billion. Culture-based studies have demonstrated chronic bacterial and/or fungal colonization of CRS patient sinus cavities implicating these species in the pathophysiology of CRS. Despite these findings, the microbiology and immunology underlying CRS remains poorly described, controversial, and to date no clear etiology has been described (*3*). Known bacterial pathogens, such as *Staphylococcus* and *Streptococcus* species isolated from CRS sinuses and implicated in the disease (*4-7*), have also been detected in the nasopharynx of healthy individuals with no sinus symptomology (*8*). These results indicate that the composition of the microbiota at discrete mucosal sites may define the abundance and pathogenic behavior of specific members of the assemblage. Such observations suggest that local microbiota composition plays a key role in protection against pathogen overgrowth and virulence gene expression, and that perturbations to local microbiota composition can contribute to infectious and inflammatory disease etiology.

[0003] The sinuses are lined with respiratory epithelia that support colonization by a diverse microbiome at upper respiratory sites, *e.g.*, oropharynx (*8, 11*). Little is known of the composition of the resident microbiome of the paranasal sinuses and the contribution of these assemblages to sinus mucosal health. Microbiological studies of this niche are based on culture-based approaches, which under-estimate the diversity of species present. The present disclosure describes results from culture-independent approaches, and thus provides a more accurate picture of sinus microbiome composition. The presently disclosed results show comparative analyses of healthy and diseased (sinusitis) samples, revealing both gross community characteristics and discrete species highly associated with health status.

BRIEF SUMMARY OF THE INVENTION

[0004] Described herein are results from a high-resolution, culture-independent comparative analysis of the sinus microbiota of chronic rhinosinusitis (CRS) patients, and healthy subjects without CRS undergoing open nasal or sinus surgery. Surgical patients typically exhibit severe disease, and surgery provides access to affected sinus mucosal surfaces that are otherwise inaccessible. The results indicate that specific features of the sinus mucosal microbiota are associated with disease state and severity, and identify both pathogenic and protective species in this niche.

[0005] Provided herein are novel probiotics for improving the sinus microbiota in an individual comprising at least one bacteria listed in Table X, *e.g.*, a Lactic Acid Bacterial (LAB) species, a Lactobacillus species, or *L. sakei*. Such probiotics can be used to treat an individual with reduced microbial diversity in the nasal sinus, or one that has been diagnosed with acute or chronic sinusitis. In some embodiments, provided is a pharmaceutical composition comprising at least one bacterial species listed in Table X, and a pharmaceutically acceptable excipient, *e.g.*, for nasal or sinus administration. In some embodiments, the composition comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, or more of the bacteria listed in Table X. In some embodiments, the composition comprises at least one LAB species. In some embodiments, the composition comprises *L. sakei*. In some embodiments, the composition consists essentially of *L. sakei*, that is, other bacterial species, if present at all, are not detectable, or not present in quantities sufficient to colonize the sinonasal mucosa.

[0006] Further provided are methods for improving the sinus microbiota (*e.g.*, increasing the microbial diversity, increasing the relative amount of beneficial bacteria such as those listed in Table X, or reducing the level of pathogenic bacteria such as Corynebacteria) in an individual comprising administering at least one bacteria listed in Table X (*e.g.*, a Lactic Acid Bacterial (LAB) species, a Lactobacillus species, or *L. sakei*); and allowing the bacteria to colonize the sinonasal mucosa. Such methods can be used to treat an individual diagnosed with sinusitis, or displaying sinusitis symptoms. In some embodiments, the administering is nasal, *e.g.*, using a spray, aerosol, syringe, irrigation, or nasal drops. In some embodiments, the maxillary sinus is colonized. In some embodiments, the ethmoid sinus is colonized. In some embodiments, the frontal sinus is colonized. In some embodiments, the sphenoid sinus is colonized.

[0007] In some embodiments, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, or more of the bacteria listed in Table X are administered. In some embodiments, at least one Lactic Acid Bacterial (LAB) species is administered. In some embodiments, *Lactobacillus sakei* is administered. In some embodiments, *L. sakei* is the only bacteria administered in an effective amount (*e.g.*, sufficient to colonize the sinonasal mucosa).

[0008] In some embodiments, the method further comprises detecting the microbial diversity of the sinonasal mucosa of the individual, before and/ or after the administering. In some embodiments, the method further comprises detecting the relative level of Corynebacteria (*e.g.*, *C. tuberculostearicum* and/or *segmentosum*) in a mucosal sample (*e.g.*, sinonasal sample) from the individual and comparing the relative level of Corynebacterium in the sample to a control of Corynebacterium levels, before and/or after the administering.

[0009] Also provided are diagnostic methods, *e.g.*, for determining if an individual has or is at risk of developing sinusitis. In some embodiments, the method of determining whether an individual has or is at risk of developing sinusitis, comprises detecting the relative level of Corynebacteria in a mucosal sample from the individual; comparing the relative level of Corynebacteria in the sample to a control of Corynebacteria levels; and determining that the individual has or is at risk of developing sinusitis where the relative level of Corynebacteria in the sample is higher than the control, wherein the control is a non-sinusitis control, or in the range of the control, wherein the control is a sinusitis-positive control. In some embodiments, the mucosal sample is from the maxillary sinus of the individual. In some embodiments, the mucosal sample is from the ethmoid, frontal, or sphenoid sinus of the individual. In some embodiments, the Corynebacteria is Corynebacterium tuberculostearicum.

[0010] In some embodiments, the method of determining whether an individual has or is at risk of developing sinusitis, comprises detecting the relative level of at least one bacteria from Table X (*e.g.*, a LAB species, *e.g., L. sakei*) in a mucosal sample from the individual; comparing the relative level of the at least one bacteria from Table X to a control of the level for the at least one bacteria; and determining that the individual has or is at risk of developing sinusitis where the relative level of the at least one bacteria from Table X in the sample is lower than the control, wherein the control is a non-sinusitis control, or in the range of the control, wherein the control is a sinusitis-positive control. In some embodiments, the mucosal sample is from the maxillary sinus of the individual. In some embodiments, the mucosal sample is from the ethmoid, frontal, or sphenoid sinus of the individual. In some embodiments, the at least one bacteria is a LAB species, *e.g.*, a Lactobacillus species.

[0011] In some embodiments, such methods further obtaining a mucosal sample from the sinonasal mucosa, *e.g.*, the maxillary sinus, of the individual before detecting. In some embodiments, a mucosal sample is taken from the ethmoid, frontal, or sphenoid sinus. In some embodiments, the method is used to confirm a diagnosis of sinusitis using an alternative method, *e.g.*, detection of elevated Muc5A expression or mucosal inflammation, or a higher than normal SNOT-20 score.

[0012] In some embodiments, such methods further comprise administering a sinusitis therapeutic agent to the individual based on a determination that the individual has or is at risk of developing sinusitis. In some embodiments, the sinusitis therapeutic is a pharmaceutical composition comprising at least one bacteria listed in Table X. In some embodiments, the pharmaceutical composition comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50 or more bacteria listed in Table X. In some embodiments, the pharmaceutical composition comprises a Lactic Acid Bacterial (LAB) species, *e.g.*, a Lactobacillus species such as *L. sakei*.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1. *Muc5A* mRNA expression (normalized to β-actin) is significantly increased in CRS patient samples compared to healthy subjects.

[0014] Figure 2. Bacterial community Richness (A), Evenness (B) and Shannon diversity (C) indices are significantly lower in CRS patient sinuses compared with healthy individuals, indicating gross perturbation to the sinus mucosal microbiota in the disease state. Figure 2D: Hierarchal cluster analysis demonstrates that the majority of healthy individuals cluster into a group distinct from CRS patients, indicating that sinus microbiome composition of healthy individuals is distinct form that of CRS patients. A heat-plot of sinus symptom severity (based on SNOT-20 scores) demonstrates that altered sinus microbiome composition is related to sinus health status.

[0015] Figure 3. Phylogenetic tree demonstrating the breadth of bacterial community members exhibiting significant positive or negative correlations with sinus symptom severity (determined by SNOT-20 score). *Corynebacterium tuberculostearicum* and *C. segmentosum* exhibited strong and significant positive correlations with severity of sinus symptomology, implicating them in disease etiology. Other community members illustrated, *e.g.*, in Table X, exhibited significant negative correlations with sinus symptomology. The results indicate that these species maintain sinus mucosal health.

[0016] Figure 4. Linear regression of Q-PCR-derived *C. tuberculostearicum* abundance with array reported florescence intensity (A) and SNOT-20 score (B) indicate strong concordance between Q-PCR and array-generated findings and confirm a strong positive relationship between *C. tuberculostearicum* abundance and symptom severity.

[0017] Figure 5. Murine experimental design. Amox= Amoxicillun; Clau= Clavulanate.

[0018] Figure 6. A. PAS stained histological sections of murine sinuses representative of animals in each treatment group treated with a combination of antibiotic and *C. tuberculostearicum*. Panel i-iv. Triplicate views of maxillary sinuses from 2 mice per treatment group were used to determine physiology (representative images are shown). Mice treated with a combination of antibiotic and *C. tuberculost*earicum show significantly increased goblet cell hyperplasia (indicated by arrows) and mucin hypersecretion compared to other treatment groups (panel iii). B. Goblet cell enumeration illustrates that, compared to other treatment groups, animals treated with a combination of antibiotic and *C. tuberculostearicum* exhibit significant increases in goblet cell number per μm of epithelium.

[0019] Figure 7. Q-PCR quantification of *C. tuberculostearium* and *L. sakei* load in murine sinus tissue. Asterisk (*) denotes statistical significance ($p<0.05$). Total 16S rRNA copy number per species was normalized to known number of 16S rRNA copies per genome for each species.

[0020] Figure 8. A. PAS stained histological sections of murine sinuses representative of animals in each treatment group at 60X magnification (panel i-v). Animals treated with a combination of antibiotic and *L. sakei* (panel iv) exhibit epithelial physiology comparable to untreated or antibiotic treated controls (panels i and ii), while animals treated with a combination of antibiotic and *C. tuberculostearicum* exhibit goblet cell hyperplasia (indicated by arrows) and mucin hypersecretion. Panel v demonstrates that mice treated with antibiotics prior to co-instillation of *C. tuberculostearicum* and *L. sakei* exhibit epithelial physiology comparable to control animals, indicating that *L. sakei* is protective against the pathogenic effects of *C. tuberculostearicum*. Triplicate views of maxillary sinuses from 2 mice per treatment group were used to determine physiology (representative images are shown). B. Enumeration of goblet cells per μm of epithelium confirms that hyperplasia is associated with a high abundance of *C. tuberculostearicum* in the sinuses. Instillation of *L. sakei* does not induce hyperplasia, and co-instillation of *L. sakei* with *C. tuberculostearicum* actually prevents the pathological response to *C. tuberculostearicum*.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[0021] Persistent mucosal inflammation and microbial infection are characteristic of Chronic Rhinosinusitis (CRS). The relationship between sinus microbiota composition and CRS is unknown. The present disclosure describes comparative microbiome profiling of a cohort of CRS patients and healthy subjects, demonstrating that the sinus microbiota of CRS patients exhibit significantly reduced bacterial diversity. Characteristic of this community collapse is the depletion of multiple, phylogenetically distinct Lactic Acid Bacteria and the concomitant increase in relative abundance of Corynebacteria, in particular, *C. tuberculostearicum*. The conditions of the human cohort were reproduced in a murine model, and confirmed the pathogenic potential of *C. tuberculostearicum*, as well as the benefit of a diverse, properly composed mucosal microbiota to protect against *C. tuberculostearicum*. Moreover, the results show that *Lactobacillus sakei*, identified from comparative microbiome analyses as protective, affords defense against *C. tuberculostearicum* sinus infection, even in the context of a depleted sinus bacterial community. The results demonstrate that sinus mucosal health depends on the local composition of resident microbiota, identifies a novel sinusitis associated pathogen, and a probiotic therapy for sinusitis.

[0022] Human health is dependent on the diverse microbial assemblages that inhabit discrete host niches, particularly mucosal-associated surfaces. To date, culture-based approaches to characterize the etiological agent of CRS have provided a reductionist and somewhat discordant view of the microbiology associated with this disease.

[0023] The culture-independent approaches described herein allow the diversity of microbiomes in specific host sites to be better characterized (*19-24*), including compositional and functional changes in disease states (*25*), and microbes highly correlated with symptom severity (*20*) or immune responses (*26*). The present results show that the composition of the resident microbiota in a given niche can strongly influence the behavior of specific species, particularly a pathogen.

[0024] A clear signal emerged from the present results demonstrating that the sinus microbiota of CRS patient cohort were characterized by both grossly depleted communities and a significant increase in relative abundance of *C. tuberculostearicum*. Though phylogenetically its closest bacterial relatives include *Mycobacteria* and *Nocardia*, genera synonymous with pathogenesis, *C. tuberculostearicum* is customarily considered an innocuous member of the healthy skin microbiota, and an unlikely etiological agent of CRS.

The presently described murine model of sinusitis confirmed both the pathogenic potential of *C. tuberculostearicum* and that its impact on sinus epithelial responses was significantly enhanced in the absence of a replete sinus microbiota. Demonstration that a bacterial species that commonly inhabits human skin, represents an etiological agent of CRS illustrates why the etiology of CRS, and likely other chronic inflammatory diseases has been so difficult to define.

[0025] The role of the microbiome, LAB species in particular, in modulating the impact of *C. tuberculostearicum* on sinus mucosal responses likely explains why it was not previously considered a pathogen. This phenomenon may also explain why, despite detection of known pathogens in the healthy subject sinus microbiota, these subjects exhibit no symptomology and, more broadly, provide an explanation as to why seemingly similar patients, exhibiting comparable quantities of known pathogenic species, may exhibit dramatically different clinical outcomes.

[0026] One advantage to the present comparative study design is the ability to identify those species associated with healthy sinuses that provide mucosal protection. The murine sinusitis model provided an opportunity to determine whether such species may afford protection against the pathophysiology induced by a combination of depleted microbiota and *C. tuberculostearicum*. The results demonstrate that *L. sakei* represents a novel probiotic therapeutic for the treatment of sinusitis sub-types, including CRS. Several members of the Lactobacillaceae, including *L. sakei*, as well as other LABs and lactic acid producing members of the Firmicutes were significantly depleted in CRS maxillary sinus. The observation indicates that these species (*e.g.*, through bacteriocin or lactic acid production (*30, 31*)) can out-compete pathogenic species, shape the sinus mucosal microbiota, and protect this niche from pathogen overgrowth.

[0027] The present results also have significant implications for the excessive use of antimicrobials, which contribute to microbiota depletion, in the treatment of viral sinusitis and other upper respiratory infections. The data suggest that microbial supplementation during periods of acute sinusitis with one or a combination of presently identified species would be more appropriate.

II. Definitions

[0028] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art. See, *e.g.*, Lackie, DICTIONARY OF CELL AND MOLECULAR BIOLOGY, Elsevier (4th ed. 2007); Sambrook *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL, Cold Springs Harbor Press (Cold Springs Harbor, NY 1989). The term "a" or "an" is intended to mean "one or more." The term "comprise" and variations thereof such as "comprises" and "comprising," when preceding the recitation of a step or an element, are intended to mean that the addition of further steps or elements is optional and not excluded. Any methods, devices and materials similar or equivalent to those described herein can be used in the practice of this invention. The following definitions are provided to facilitate understanding of certain terms used frequently herein and are not meant to limit the scope of the present disclosure.

[0029] The term "colonize" in the context of the present disclosure refers to microbial growth and expansion. For example, a population of bacteria can be said to colonize an environment if it survives in the environment through at least one round of cell division.

[0030] The term "bacterial load" refers to a measure of the total number of bacteria in a given environment, *e.g.*, an airway mucosal surface. The bacterial load is typically expressed as colony forming units (CFU) per ml, per gram of sample or tissue, or per surface area.

[0031] The term "microbiome" refers to the community of microbes and environmental interactions in a defined environment. The microbiome also includes the genetic makeup of the microbes. Mucosal surfaces are examples of microbiomes in animals, *e.g.*, the gut, airways, sinus, vagina, oral cavity, etc. Skin, and particular skin areas, comprise additional microbiomes. The term is derived from the term "biome" which can be used synonymously with ecosystem to refer to a larger scale environment and its inhabitants.

[0032] The term "improving the sinus microbiota" refers to increasing microbial diversity, increasing the relative amount of beneficial bacteria (*e.g.*, bacteria listed in Table X), or reducing the relative amount of bacteria with high levels in sinusitis patients (*e.g.*, Corynebacteria).

[0033] Microbial diversity refers to the range of different species or strains present in a sample. The sample can be highly diverse, with a comparatively wide range of taxa, or lacking in diversity, *e.g.*, with comparatively few taxa represented compared to a normal control. Microbial diversity can be expressed in general comparative terms (e.g., more or less

diverse, compared to a normal, healthy control). Microbial diversity can also be expressed in absolute numbers or ranges of numbers, *e.g.*, more than 1000 species or strains, or 500-100, 50-100 or, 25-50 species or strains, etc. in a given environment.

[0034] The amount of a particular strain or species of bacteria can be expressed in absolute numbers or ranges of numbers, *e.g.*, 10^6 or 10^6-10^7 Lactobacillus in a sample, or in a given volume. The amount can also be expressed in terms of colony forming units, or absorbance, depending on the assay used for bacterial detection, as will be appreciated by one of skill. The amount can also be expressed in comparative terms, *e.g.*, compared to a control. The relative level of a given strain or species refers to the amount relative to other strains or species within the sample. For example, a high relative level of a Corynebacterial strain indicates that the amount of Corynebacterial strain, as a percentage of total microbes, is higher than that of a normal control. This can be due to depletion of other species in the microbiome, or because of high levels of Corynebacteria.

[0035] "Lactic acid bacteria" (LAB) refers to species that fall into the order of Gram-positive Lactobacillalese. LAB genera include: *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, and *Streptococcus*, as well as *Aerococcus*, *Carnobacterium*, *Enterococcus*, and *Tetragenococcus*.

[0036] A "control" sample or value refers to a sample that serves as a reference, usually a known reference, for comparison to a test sample. For example, a test sample can be taken from a patient suspected of having sinusitis and compared to samples from a known sinusitis patient (positive or disease control) or a known normal (negative, non-sinusitis, non-disease, or healthy control) individual. A control can also represent an average range or value gathered from a population of similar individuals, *e.g.*, sinusitis patients or healthy individuals with a similar medical background, same age, weight, etc. A control value can also be obtained from the same individual, *e.g.*, from an earlier-obtained sample, prior to disease, or prior to treatment. One of skill will recognize that controls can be designed for assessment of any number of parameters.

[0037] One of skill in the art will understand which controls are valuable in a given situation and be able to analyze data based on comparisons to control values. Controls are also valuable for determining the significance of data. For example, if values for a given parameter are widely variant in controls, variation in test samples will not be considered as significant.

[0038] "Subject," "patient," "individual" and like terms are used interchangeably and refer to, except where indicated, humans and non-human animals. The term does not necessarily indicate that the subject has been diagnosed with a particular disease, but typically refers to an individual under medical supervision. A patient can be an individual that is seeking treatment, monitoring, adjustment or modification of an existing therapeutic regimen, etc.

[0039] The term "in the range of the control" refers to a value that falls within the range of control values for a given control condition. Where one value is given as a control, "in the range of the control" refers to a value that is not statistically different from the control as determined by one of skill in the art. For example, within the range of the control can be ± 5%, 10%, 20%, or within 0.5-fold, 1-fold, or 2-fold difference from a control value at a given condition. One of skill will understand that corrective calculations can be made, *e.g.*, to account for age, severity of condition, antibiotic use, etc.

[0040] As used herein, the term "pharmaceutically acceptable" is used synonymously with physiologically acceptable and pharmacologically acceptable with respect to, e.g., a pharmaceutical composition. A pharmaceutical composition will generally comprise agents for buffering and preservation in storage, and can include buffers and carriers for appropriate delivery, depending on the route of administration.

[0041] The terms "dose" and "dosage" are used interchangeably herein. A dose refers to the amount of active ingredient given to an individual at each administration. For the present invention, the dose will generally refer to the amount of probiotic, antibiotic or anti-inflammatory agent. Dosage can also be expressed in terms of bacterial concentration. The dose will vary depending on a number of factors, including frequency of administration; size and tolerance of the individual; severity of the condition; risk of side effects; and the route of administration. One of skill will recognize that the dose can be modified depending on the above factors or based on therapeutic progress. The term "dosage form" refers to the particular format of the pharmaceutical, and depends on the route of administration. For example, a dosage form can be in a liquid form for nebulization, *e.g.*, for inhalants, in a tablet or liquid, *e.g.*, for oral delivery, or a saline solution, *e.g.*, for injection.

[0042] As used herein, the terms "treat" and "prevent" are not intended to be absolute terms. Treatment can refer to any delay in onset, reduction in the frequency or severity of symptoms, amelioration of symptoms, improvement in patient comfort and/or respiratory function, etc. The effect of treatment can be compared to an individual or pool of individuals

not receiving a given treatment, or to the same patient prior to, or after cessation of, treatment.

[0043] The term "prevent" refers to a decrease in the occurrence of sinusitis symptoms in a patient. As indicated above, the prevention may be complete (no detectable symptoms) or partial, such that fewer symptoms are observed than would likely occur absent treatment.

[0044] The terms "effective amount" and "therapeutically effective amount," refer to that amount of a therapeutic agent sufficient to ameliorate the target disorder. For example, for a given disease parameter, a therapeutically effective amount will show an increase or decrease of at least 5%, 10%, 15%, 20%, 25%, 40%, 50%, 60%, 75%, 80%, 90%, or at least 100%. Therapeutic efficacy can also be expressed as "-fold" increase or decrease. For example, a therapeutically effective amount can have at least a 1.2-fold, 1.5-fold, 2-fold, 5-fold, or more effect over a control.

[0045] The term "diagnosis" refers to a relative probability that a disease is present in the subject. Similarly, the term "prognosis" refers to a relative probability that a certain future outcome may occur in the subject. The terms are not intended to be absolute, as will be appreciated by one of skill in the field of medical diagnostics.

[0046] "Nucleic acid" or "oligonucleotide" or "polynucleotide" or grammatical equivalents used herein means at least two nucleotides covalently linked together. Oligonucleotides are typically from about 5, 6, 7, 8, 9, 10, 12, 15, 25, 30, 40, 50 or more nucleotides in length, up to about 100 nucleotides in length. Nucleic acids and polynucleotides are a polymers of any length, including longer lengths, *e.g.*, 200, 300, 500, 1000, 2000, 3000, 5000, 7000, 10,000, etc. A nucleic acid of the present invention will generally contain phosphodiester bonds, although in some cases, nucleic acid analogs are included that may have alternate backbones, comprising, *e.g.*, phosphoramidate, phosphorothioate, phosphorodithioate, or O-methylphophoroamidite linkages (see Eckstein, Oligonucleotides and Analogues: A Practical Approach, Oxford University Press); and peptide nucleic acid backbones and linkages. Other analog nucleic acids include those with positive backbones; non-ionic backbones, and non-ribose backbones, including those described in U.S. Patent Nos. 5,235,033 and 5,034,506, and Chapters 6 and 7, ASC Symposium Series 580, *Carbohydrate Modifications in Antisense Research*, Sanghui & Cook, eds. Nucleic acids containing one or more carbocyclic sugars are also included within one definition of nucleic acids. Modifications of the ribose-phosphate backbone may be done for a variety of reasons, *e.g.*, to increase the stability and half-life of such molecules in physiological environments or as probes on a biochip.

Mixtures of naturally occurring nucleic acids and analogs can be made; alternatively, mixtures of different nucleic acid analogs, and mixtures of naturally occurring nucleic acids and analogs may be made.

[0047] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers, those containing modified residues, and non-naturally occurring amino acid polymer.

[0048] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function similarly to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *e.g.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs may have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions similarly to a naturally occurring amino acid.

[0049] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0050] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical or associated, e.g., naturally contiguous, sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode most proteins. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to another of the corresponding codons described without altering the encoded

polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes silent variations of the nucleic acid. One of skill will recognize that in certain contexts each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, often silent variations of a nucleic acid which encodes a polypeptide is implicit in a described sequence with respect to the expression product, but not with respect to actual probe sequences.

[0051] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.typically conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (*see, e.g.,* Creighton, *Proteins* (1984)).

[0052] The terms "label," "tag," "detectable moiety," etc. refer to compositions or components that are detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, useful labels include ^{32}P, fluorescent dyes, electron-dense reagents, enzymes (*e.g.*, as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins or other entities which can be made detectable, *e.g.*, by incorporating a radiolabel into a peptide or antibody specifically reactive with a target peptide. Any method known in the art for conjugating an antibody to the label may be employed, *e.g.*, using methods described in Hermanson, Bioconjugate Techniques 1996, Academic Press, Inc., San Diego.

[0053] A "labeled nucleic acid probe or oligonucleotide" is one that is bound, either covalently, through a linker or a chemical bond, or noncovalently, through ionic, van der Waals, electrostatic, or hydrogen bonds to a label such that the presence of the probe may be detected by detecting the presence of the label bound to the probe. Alternatively, method

using high affinity interactions may achieve the same results where one of a pair of binding partners binds to the other, *e.g.*, biotin, streptavidin.

[0054] The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence with a higher affinity, *e.g.*, under more stringent conditions, than to other nucleotide sequences (*e.g.*, total cellular or library DNA or RNA). One of skill in the art will appreciate that specific hybridization between nucleotides usually relies on Watson-Crick pair bonding between complementary nucleotide sequences.

[0055] The term "probe" or "primer", as used herein, is defined to be one or more nucleic acid fragments whose specific hybridization to a sample can be detected. A probe or primer can be of any length depending on the particular technique it will be used for. For example, PCR primers are generally between 10 and 40 nucleotides in length, while nucleic acid probes for, *e.g.*, a Southern blot, can be more than a hundred nucleotides in length. The probe may be unlabeled or labeled as described below so that its binding to the target or sample can be detected. The probe can be produced from a source of nucleic acids from one or more particular (preselected) portions of a chromosome, *e.g.*, one or more clones, an isolated whole chromosome or chromosome fragment, or a collection of polymerase chain reaction (PCR) amplification products. The length and complexity of the nucleic acid fixed onto the target element is not critical to the invention. One of skill can adjust these factors to provide optimum hybridization and signal production for a given hybridization procedure, and to provide the required resolution among different genes or genomic locations.

[0056] The probe may also be isolated nucleic acids immobilized on a solid surface (*e.g.*, nitrocellulose, glass, quartz, fused silica slides), as in an array. In some embodiments, the probe may be a member of an array of nucleic acids as described, for instance, in WO 96/17958. Techniques capable of producing high density arrays can also be used for this purpose (*see*, *e.g.*, Fodor (1991) *Science* 767-773; Johnston (1998) *Curr. Biol.* 8: R171-R174; Schummer (1997) *Biotechniques* 23: 1087-1092; Kern (1997) *Biotechniques* 23: 120-124; U.S. Patent No. 5,143,854). One of skill will recognize that the precise sequence of the particular probes described herein can be modified to a certain degree to produce probes that are "substantially identical" to the disclosed probes, but retain the ability to specifically bind to (*i.e.*, hybridize specifically to) the same targets or samples as the probe from which they were derived. Such modifications are specifically covered by reference to the individual probes described herein.

[0057] The term "antibody" refers to a polypeptide structure, *e.g.*, an immunoglobulin, conjugate, or fragment thereof that retains antigen binding activity, *e.g.*, for a bacterial antigen. The term includes but is not limited to polyclonal or monoclonal antibodies of the isotype classes IgA, IgD, IgE, IgG, and IgM, derived from human or other mammalian cells, including natural or genetically modified forms such as humanized, human, single-chain, chimeric, synthetic, recombinant, hybrid, mutated, grafted, and *in vitro* generated antibodies. The term encompases conjugates, including but not limited to fusion proteins containing an immunoglobulin moiety (*e.g.*, chimeric or bispecific antibodies or scFv's), and fragments, such as Fab, F(ab')2, Fv, scFv, Fd, dAb and other compositions.

[0058] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. Typically, the "variable region" contains the antigen-binding region of the antibody (or its functional equivalent) and is most critical in specificity and affinity of binding. See Paul, *Fundamental Immunology* (2003). The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

[0059] Antibodies can exist as intact immunoglobulins or as any of a number of well-characterized fragments produced by digestion with various peptidases. Pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the $F(ab)'_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (*see Fundamental Immunology* (Paul ed., 3d ed. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (*e.g.*, single chain Fv) or those identified using phage display libraries (*see, e.g.,* McCafferty *et al.*, *Nature* 348:552-554 (1990)).

[0060] As used herein, "V-region" refers to an antibody variable region domain comprising the segments of Framework 1, CDR1, Framework 2, CDR2, and Framework 3, including CDR3 and Framework 4, which segments are added to the V-segment as a consequence of

rearrangement of the heavy chain and light chain V-region genes during B-cell differentiation.

[0061] As used herein, "complementarity-determining region (CDR)" refers to the three hypervariable regions in each chain that interrupt the four "framework" regions established by the light and heavy chain variable regions. The CDRs are primarily responsible for binding to an epitope of an antigen. The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3, numbered sequentially starting from the N-terminus, and are also typically identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 is located in the variable domain of the heavy chain of the antibody in which it is found, whereas a V_L CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found.

[0062] The terms "antigen," "immunogen," "antibody target," "target analyte," and like terms are used herein to refer to a molecule, compound, or complex that is recognized by an antibody, *i.e.*, can be specifically bound by the antibody. The term can refer to any molecule that can be specifically recognized by an antibody, *e.g.*, a polypeptide, polynucleotide, carbohydrate, lipid, chemical moiety, or combinations thereof (*e.g.*, phosphorylated or glycosylated polypeptides, etc.). One of skill will understand that the term does not indicate that the molecule is immunogenic in every context, but simply indicates that it can be targeted by an antibody. Examples of target antigens in the context of the present disclosure include bacterial antigens (*e.g.*, from *C. tuberculostearicum*, *L. sakei*, or an LAB species) and disease-associated antigens, *e.g.*, Muc5A.

[0063] Antibodies bind to an "epitope" on an antigen. The epitope is the localized site on the antigen that is recognized and bound by the antibody. Epitopes can include a few amino acids or portions of a few amino acids, *e.g.*, 5 or 6, or more, *e.g.*, 20 or more amino acids, or portions of those amino acids. In some cases, the epitope includes non-protein components, *e.g.*, from a carbohydrate, nucleic acid, or lipid. In some cases, the epitope is a three-dimensional moiety. Thus, for example, where the target is a protein, the epitope can be comprised of consecutive amino acids, or amino acids from different parts of the protein that are brought into proximity by protein folding (*e.g.*, a discontinuous epitope). The same is true for other types of target molecules that form three-dimensional structures.

III. Diagnostic methods

[0064] The present results reveal that sinusitis patients have greatly reduced microbial diversity in the sinonasal environment (*e.g.*, maxillary sinus). In addition, Corynebacterial species (*e.g.*, *C. tuberculostearicum* and *segmentosum*) are present at a higher level than in normal healthy sinus mucosa, and correlate strongly with symptom severity. Sinusitis can also be diagnosed, *e.g.*, by detecting mucin levels, mucosal inflammation, or with self-reporting of sinus inflammation symptoms. Diagnostic methods can be used alone or in any combination.

[0065] Patients can thus be selected for therapy based on determination of microbial diversity, reduced levels of beneficial probiotic species (*e.g.*, those listed in Table X, Lactic Acid Bacteria, or *L. sakei* in particular), or elevated levels of Corynebacteria. Anatomical characterization, such as characterization of the sinus mucosal lining, can be accomplished using standard imaging techniques. Bacteria can be detected using nucleic acid techniques as described herein (*e.g.*, arrays, hybridization, or PCR), using sequences complementary to species- or order-specific nucleic acid sequences. Antibodies specific for particular bacterial species can also be utilized.

[0066] Methods of obtaining a mucosal sample from an individual are known in the art. Such methods include swabbing or brushing the sinonasal mucosa, *e.g.*, using anesthetic or endoscopic methods if necessary. In some cases, a mucosal biopsy is taken. Samples can also be obtained using a nasal lavage or spray in sufficient volume to obtain sample from the appropriate location. Comparison of various sample gathering techniques is described, *e.g.*, in Roediger *et al.* (2010) *Am J Rhinol Allergy* 24:263.

[0067] A diagnostic agent specific for the microbes described herein can include any label as known in the art, for example, in the following references: Armstrong *et al.*, *Diagnostic Imaging*, 5th Ed., Blackwell Publishing (2004); Torchilin, V. P., Ed., *Targeted Delivery of Imaging Agents*, CRC Press (1995); Vallabhajosula, S., *Molecular Imaging: Radiopharmaceuticals for PET and SPECT*, Springer (2009). A diagnostic agent can be detected by a variety of ways, including as an agent providing and/or enhancing a detectable signal. Detectable signals include, but are not limited to, fluorescent, luminescent, gamma-emitting, radioactive, optical, absorptive, etc. The terms "detectable agent," "detectable moiety," "label," "imaging agent," and like terms are used synonymously herein.

[0068] A radioisotope can be incorporated into the diagnostic agents described herein and can include radionuclides that emit gamma rays, positrons, beta and alpha particles, and X-

rays. Suitable radionuclides include but are not limited to ^{225}Ac, ^{72}As, ^{211}At, ^{11}B, ^{128}Ba, ^{212}Bi, ^{75}Br, ^{77}Br, ^{14}C, ^{109}Cd, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{3}H, ^{166}Ho, ^{123}I, ^{124}I, ^{125}I, ^{130}I, ^{131}I, ^{111}In, ^{177}Lu, ^{13}N, ^{15}O, ^{32}P, ^{33}P, ^{212}Pb, ^{103}Pd, ^{186}Re, ^{188}Re, ^{47}Sc, ^{153}Sm, ^{89}Sr, ^{99m}Tc, ^{88}Y and ^{90}Y.

[0069] In some embodiments, the diagnostic agent can be associated with a secondary binding ligand or to an enzyme (an enzyme tag) that will generate a colored product upon contact with a chromogenic substrate. Examples of suitable enzymes include urease, alkaline phosphatase, (horseradish) hydrogen peroxidase and glucose oxidase. Secondary binding ligands include, *e.g.*, biotin and avidin or streptavidin compounds as known in the art.

[0070] In some embodiments, the diagnostic agents can include optical agents such as fluorescent agents, phosphorescent agents, chemiluminescent agents, and the like. Numerous agents (*e.g.*, dyes, probes, labels, or indicators) are known in the art and can be used in the present invention. (*See, e.g.*, Invitrogen, The Handbook—A Guide to Fluorescent Probes and Labeling Technologies, Tenth Edition (2005)). Fluorescent agents can include a variety of organic and/or inorganic small molecules or a variety of fluorescent proteins and derivatives thereof. For example, fluorescent agents can include but are not limited to cyanines, phthalocyanines, porphyrins, indocyanines, rhodamines, phenoxazines, phenylxanthenes, phenothiazines, phenoselenazines, fluoresceins, benzoporphyrins, squaraines, dipyrrolo pyrimidones, tetracenes, quinolines, pyrazines, corrins, croconiums, acridones, phenanthridines, rhodamines, acridines, anthraquinones, chalcogenopyrylium analogues, chlorins, naphthalocyanines, methine dyes, indolenium dyes, azo compounds, azulenes, azaazulenes, triphenyl methane dyes, indoles, benzoindoles, indocarbocyanines, benzoindocarbocyanines, and BODIPY™ derivatives.

IV. Methods and compositions for administration

[0071] Provided herein are methods of improving sinus microbiota (*e.g.*, increasing microbial diversity or reducing the relative population of pathogenic species) in an individual comprising administering a pharmaceutical composition comprising a beneficial microbe, *e.g.*, from Table X, to the individual. Typically, the composition is administered nasally, trans-nasally, or to the sinuses, *e.g.*, using an aerosol, spray, irrigation, or nasal drops. Such methods can be used to treat an individual diagnosed with sinusitis.

[0072] A pharmaceutical composition comprising a beneficial microbe described herein can be administered, alone or in combination with other suitable components, using an

aerosol formulations ("nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, etc.

[0073] Compositions for administration typically comprises at least one probiotic microbe, *e.g.*, as identified in Table X, in a pharmaceutically acceptable carrier, *e.g.*, an aqueous carrier. A variety of aqueous carriers can be used, *e.g.*, buffered saline. These solutions are generally free of undesirable matter, *e.g.*, contaminating species. The compositions can contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of active agent in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs.

[0074] The formulation may also provide additional active compounds, including, *e.g.*, antibiotic or anti-inflammatory agents. Combination therapies contemplate coadministration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order.

[0075] The active ingredients can be prepared as sustained-release preparations (*e.g.*, semi-permeable matrices of solid hydrophobic polymers (*e.g.*, polyesters, hydrogels (for example, poly (2-hydroxyethyl-methacrylate), or poly (vinylalcohol)), polylactides. The probiotic formulations described herein can also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin microcapsules and poly- (methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions.

[0076] Methods for preparing therapeutic compositions will be known to those skilled in the art and are described in more detail in such publications as Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa. (1980).

[0077] The compositions can be administered for therapeutic or prophylactic treatments. In therapeutic applications, compositions are administered to a patient suffering from a disease (*e.g.*, sinusitis) in an effective dose. Amounts effective for this use will depend upon the route of administration, the severity of the condition, and the general state of the patient's

health. Single or multiple administrations of the compositions may be administered depending on the dosage and frequency as required and tolerated by the patient. The presently described compositions can be administered to humans and other animals. Thus the methods are applicable to both human therapy and veterinary applications.

[0078] To determine a therapeutically effective dose, *e.g.* a colonizing dose of a probiotic composition, a relatively low dose of the composition can be initially administered to the individual, and the dose can be incrementally increased until the condition of the individual, *e.g.*, the sinus environment, begins to improve (*e.g.*, reduced mucin secretion or goblet cell hyperplasia, or increased microbial diversity). In some cases, however, the initial dose is relatively high to establish a colonizing population in a patient experiencing acute symptoms. One of skill will appreciate that a number of variables must be considered when determining a therapeutically effective dose. The dose administered to a patient should be sufficient to effect a beneficial therapeutic response in the patient over time. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular composition (or combination therapy) in a particular patient.

V. Kits

[0079] Further provided are kits for detecting at least one sinusitis associated bacterial taxa (*i.e.*, bacteria with high levels correlating with sinusitis, or bacteria are associated with the absence of sinusitis, such as the bacteria in Table X). The kit can optionally include written instructions, reference to an internet site, or electronic instructions (*e.g.*, on a CD-ROM or DVD). In some embodiments, kits of the invention will include a case or container for holding the reagents in the kit, which can be included separately or in combination. In some embodiments, the kit includes brushes, swabs, or other sample gathering devices for obtaining a sinonasal sample from an individual, *e.g.*, from the maxillary, ethmoid, frontal, and/or sphenoid sinus. The kit can further comprise sample containers for holding and processing samples, and for detection of sinonasal microbes in the sample.

[0080] In some embodiments, the kit includes an array, the array comprising probes or other agents capable of specific detection of at least one sinusitis associated bacterial taxa. For example, the array can comprise probes that specifically hybridize to nucleotide sequences from the at least one sinusitis associated bacteria, *e.g.*, polynucleotide or oligonucleotide probes. In some embodiments, the array comprises components (*e.g.*, antibody fragments) that specifically detect non-nucleic acid markers from the at least one

sinusitis associated bacteria. In some embodiments, the array can specifically detect at least 4, 5, 10, 12, 15, 20, 50, 80, 100, or all of the sinusitis associated bacteria, *e.g.*, Corynebacteria or those listed in Table X. In some embodiments, the kit further includes reagents (buffers, secondary detection agents, etc.) required for running the detection reaction.

[0081] In some embodiments, the kit will include PCR reagents and primers for detecting at least one of the bacteria disclosed in Table X, or at least one Corynebacterial species. The kit can thus include a polymerase, nucleotide monomers, buffer stocks, and optionally an interchalating fluorescent dye. In some embodiments, the kit can include PCR primers for at least 4, 5, 10, 12, 15, 20, 50, 60, or all of the sinusitis associated bacteria, e.g., Corynebacteria or those listed in Table X.

[0082] In some embodiments, the kit can include PCR primers for detecting a polynucleotide from at least one sinusitis associated bacteria, *e.g.*, Corynebacteria (*e.g.*, tuberculostearicum or segmentosum), or at least one bacteria listed in Table X. The primers can be designed to amplify more than one species or strain from a bacterial taxa, *e.g.*, more than one Corynebacteria or Lactobacillus strain.

[0083] In some embodiments, the kit will include a positive control for the at least one sinusitis associated bacteria (*e.g.*, an isolated sample of the bacteria). In some embodiments, the kit will include a negative control for the at least one sinusitis associated bacteria (*e.g.*, a blank, or an unrelated bacterial sample).

[0084] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, patent applications, Accession or ID numbers, and websites cited herein are hereby incorporated by reference in their entirety for all purposes.

VI. Examples

A. Materials and methods

[0085] ***Patient sample collection.*** Patient and disease stratification was performed based on recent clinical history, nasal endoscopy, CT sinus review, and a validated quality-of-life instrument, the disease-specific Sinonasal Outcome Test survey [SNOT-20; (*38*)]. Sinus

brushings were obtained during functional endoscopic sinus surgery (FESS) of CRS patients, or surgery for non-CRS complaints, *e.g.*, obstructive sleep apnea or post-traumatic malocclusion, in healthy subjects. Endoscopically-guided brush samples of mucosal surfaces of the lateral, central, and medial portions of the maxillary sinus were obtained and pooled together in 1 ml of RNALater for analysis. Samples were placed at 4 °C for 24 hours prior to storage at -80 °C until processed.

[0086] ***DNA extraction and PhyloChip analysis.*** Mucosal brushings were transferred to Lysis Matrix B (MP Biomedicals, OH) tubes containing 600ul RLT buffer (Qiagen, CA). Samples were subjected to 30 seconds of bead beating at 5.5 m/second followed by centrifugation for one minute at 2000 rpm. Supernatant was transferred to the AllPrepDNA spin column and nucleic acid purification (DNA and RNA) was carried out according to the manufacturer's instructions (Qiagen, CA). Nucleic acid concentrations were determined using a NanoDrop spectophotometer (Thermo Scientific, DE). PhyloChip analysis was performed as previously described (*40*) using 250 ng of purified pooled 16S rRNA amplicon per sample generated using 27F and 1492R universal primers (*41*). Data sets were conservatively filtered as previously described (*40*). Probe-set fluorescence intensity was normalized and log transformed prior to analysis using packages in the R statistical environment. Hierarchical cluster analysis (HCA) was performed on a Bray-Curtis dissimilarity matrix generated from PhyloChip fluorescence intensity data using the vegan package as we have previously described (*22*), followed by average linkage clustering.

[0087] A two-tailed Welch's T-test was used to identify taxa that were significantly altered in relative abundance across healthy subject and patient groups and adjusted for false discovery (*42*) using the q-value package as previously described at the website accessible at cran.r-project.org/web/packages/qvalue/qvalue.pdf. Results with a p-value < 0.05 and q-value < 0.05, were considered statistically significant. The 16S rRNA sequences of significant taxa were used to construct a neighbor-joining with nearest-neighbor interchange tree using FastTree (*43*) which was annotated using the Interactive Tree of Life website accessible at itol.embl.de (*44*).

[0088] ***Murine sinus studies.*** Female 4-5 week old C57BL6/J mice weighing 16 to 18 g were purchased form Jackson Laboratories (Bar Harbor, ME) and housed in micro-isolator cages. Animals were permitted to acclimatize for two weeks with food and water *ad libitum* prior to study. Mice were administered augmentin (amoxicillin/clavulanate) at 100 mg/kg dosage once a day for five days prior to bacterial instillation. Prior to intra-nasal *C. tuberculostearicum, L. sakei*, or combination of both *C. tuberculostericum* and *L. sakei*,

inoculations mice were anesthetized by intraperitoneal injection with avertin (250 mg/kg). Inoculation was performed once a day for three days by applying 25 µl of either *C. tuberculstearicum* suspension (1.0 X 10^{11} cfu/ml) or *L. sakei* (1.0 X 10^{11} cfu/ml) suspension, or combination of both microbes in equal ratios (total 2.0 X 10^{11} cfu/ml), in PBS onto the external nares and inhalation by the animals (*see e.g., 45, 46*). Mice were monitored for breathing during the entire inoculation process and post inoculation until fully recovered from anesthesia. Twenty-four hours after the final bacterial inoculation, mice were euthanized by CO_2 asphyxiation followed by induced pneumothorax. The heads were decapitated and sinuses dissected for histological (n = 2 animals per treatment group) and molecular analysis (n = 3 animals per treatment group). Dissected sinuses used for molecular analyses were placed in RNAlater (Ambion, TX) and stored at 4°C until processed the following day in the same manner as described above for human samples.

[0089] ***Sinus histology***. Sinuses used for histological analysis were fixed overnight in 4% paraformaldehye, followed by overnight decalcification in Decalcifying solution A (Fisher, CA). Samples were then dehydrated as follows: rinsed with PBS (1 hour), soaked in 30% ethanol (1 hour), 50% ethanol (1 hour), then transferred to 70% ethanol and stored at 4 °C until subsequent preparation. Sinus samples were sectioned to 5 µm thickness and mounted onto glass microscope slides. H&E and PAS staining were performed as previously described (*47*). PAS-stained sections prepared from four groups of mice (CT-AB-, CT-AB+, CT+AB- and CT+AB-) were imaged at 20X and 60X magnifications, and PAS-positive cells were counted for 3 different sections per mice (2 mice in each group). The number of goblet cells was expressed as the total number of PAS-positive cells per µm length of epithelium. Students t-test was used to calculate p-values, $p < 0.05$ was considered significant.

[0090] ***Q-PCR.*** Bacterial burden was determined using extracted DNA (10 ng per sample, triplicate reactions) and universal 16S rRNA Q-PCR primers [338F, 5'-ACTCCTACGGGAGG CAGCAG -3' (*41*) and 518R 5'- ATTACCGCGGCTGCTGG -3'(*48*)]. Quantitec SYBR Green (Qiagen, CA) was used according to manufacturer's instructions. Reaction mixtures (25 µl total) contained 12.5 µl of 2X QuantiTec SYBR green (Qiagen, CA), 2.5 µl each of 3 µM forward and reverse primer, and 6.5 µl H_2O. Reactions were amplified using the Mx3000P Real-Time PCR System (Stratagene, CA) and the following cycling conditions: 95 °C for 10 min followed by 40 cycles of 95 °C for 30 sec, 55 °C for 1 min and 72 °C for 30 sec. The data acquisition step was set at 55 °C. *Corynebacteriumtuberculostearicum* abundance was determined by Q-PCR using a pair of primers designed in this study: CT-F: 5'-GAACGGAAAGGCCCTGCTTGCA-3' and CT-R

5'-GGCTCCTATCCGGTAT TAGACC-3'; *Lactobacillus sakei* abundance was determined using the primer pair: LS-F: 5'-GGTAAAGGCTCACCAAGACCGTGAT-3' and LS-R: 5'TCACGCGGCGTTGCTCCATC-3'. Reaction mixtures (25 µl total) contained 10 ng of total DNA, 12.5 µl of 2X QuantiTec SYBR green (Qiagen, CA), 2.5 µl of each primer and 6.5 µl of H_2O. Reactions were amplified on the Mx3000P Real-Time PCR System (Stratagene, CA) under the following steps: 95 °C for 10 min followed by 40 cycles of 95 °C for 30 sec, 55 °C for 1 min and 72 °C for 30 sec. The data acquisition step was set at 55 °C.

[0091] For *Muc5A* expression analysis, confirmed DNA-free total RNA (1 µg) was reverse transcribed at 42 °C for 50 min in a 20 µl reaction mixture (0.025 µg/µl oligo-dT, 0.5 mMdNTPs, 5µl First Strand buffer, 5 mM MgCl2, 0.01 M DTT, and RNAse Out) using 1 µl Superscript II (Invitrogen, CA). cDNA was diluted 1:5 in molecular grade water. Reactions were performed in 96 well plates in a 25 µl final volume containing 12.5 µl of SYBR Green PCR master mix (Qiagen, CA), 2.5 µl of each forward and reverse primer (final concentration 10 µM per primer; Muc5AF 5'- TGTGGCGGGAAAGACAGC-3' Muc5AR 5'-CCTTCCTATGGCTTAGCTTCAGC-3'; β-actinF 5'- CACCACACCTTCTAC AATGAGC TGC-3' and β-actinR 5'- ACACCCTGGATAGCAACGTACATGC-3'), 4 µl of diluted cDNA, and 6 µl of molecular grade water. Reactions were amplified on a Mx3000P Real-Time PCR System under the following conditions: 94 °C for 10 min followed by 40 cycles of 94 °C for 30 sec, 58 °C for 1 min and 72 °C for 30 sec. Students t-test was used to calculate p-values for individual treatment groups, $p < 0.05$ was considered significant.

B. Example 1: Comparative sinus microbiota analysis

[0092] Maxillary sinus samples from 20 subjects (10 CRS and 10 healthy individuals) were used for this study. Patient details are provided in Table 1. Mucin hyper-secretion is a hallmark of sinus disease (*12, 13*). Therefore, to confirm that the CRS patients exhibited a phenotype consistent with disease, Q-PCR analysis of *Muc5A* gene (encoding mucin primarily secreted from surface epithelium goblet cells in humans (*14, 15*)) expression was performed. *Muc5A* was significantly up-regulated in CRS patients compared to healthy control subjects (Figure 1), thus validating the presence of sinus disease in our CRS cohort. Q-PCR analysis of bacterial burden by total 16S rRNA copy number demonstrated that peri-operative CRS patients and healthy subjects exhibited no significant difference in sinus bacterial burden ($2.19 \times 10^6 \pm 1.09 \times 10^6$ vs $2.86 \times 10^6 \pm 2.34 \times 10^6$copies of 16S rRNA gene per µg of total DNA in CRS and healthy control subjects respectively; $p \leq 0.53$). The result indicates that the sinus niche can support a defined bacterial load, and that microbiota

composition and relative taxonomic distribution, rather than absolute number of bacteria present, are related to disease state.

Table 1. Patient information.

Group	Study ID	Gender	Age	SNOT-20	Antimicrobial treatment	
					Pre-operative	Peri-operative
CRS	CRS-001[a]	M	54	2.75	None[b]	Cefazolin
CRS	CRS-002	M	54	2.35	TMP/SMX[c]	Vancomycin
CRS	CRS-003[a]	M	48	2.65	None	AMP/SUL[d]
CRS	CRS-004	M	33	3.60	None	Cefazolin
CRS	CRS-005[a]	M	56	2.65	Clarithromycin	Cefazolin
CRS	CRS-006	F	41	1.85	None	Cefazolin
CRS	CRS-007	M	60	3.00	Clarithromycin	Levofloxacin
CRS	CRS-008	M	53	1.55	Ciprofloxacin	Cefazolin
CRS	CRS-009	F	46	2.35	AMO/CLU[e]	Cefazolin
Control	CRS-010	M	62	0.90	Levofloxacin	Centriaxone
CRS	CRS-011	M	42	3.35	AMO/CLU	Clindamycin
Control	CRS-012[a]	M	43	0.15	None	Clindamycin
Control	CRS-013	F	73	1.70	None	Cefazolin
Control	CRS-014	M	41	2.60	None	Clindamycin
Control	CRS-015[a]	M	39	0.00	None	Clindamycin
Control	CRS-016[a]	F	37	2.25	None	Cefazolin
Control	CRS-017	F	46	0.10	None	Clindamycin
Control	CRS-018	M	46	2.15	None	Clindamycin
Control	CRS-019	F	31	0.50	None	Clindamycin
Control	CRS-020	F	18	0.30	None	Clindamycin

[a]Microbiota profiling was not preformed for these subject samples due to insufficient 16S rRNAamplicon; [b] None, no antibiotics administered; [c]TMP/SMX, Trimethoprim/Sulfamethoxazole; [d]AMP/SUL, Ampicillin/Sulbactam; [e]AMO/CLU, Amoxicillin/ clavulanate.

[0093] A standardized phylogenetic microarray, the 16S rRNAPhyloChip was used to profile the presence and relative abundance of approximately 8,500 bacterial taxa, representing broad membership of all known bacterial phyla (Hugenholtz phylogenetic classification). This approach was used in favor of more traditional sequencing approaches to maximize the depth of community coverage. Low abundance microbiome members can contribute considerably to microbiome function (*16*) and act as keystone species that shape microbial community composition(*17*). Using this tool, we profiled bacterial communities present in 14 subjects (7 healthy, 7 CRS) with sufficient amplified 16S rRNA product to be analyzed. Comparative analyses of gross bacterial community metrics between the CRS and healthy groups demonstrated that compared to healthy individuals, CRS patients exhibited substantial microbiota perturbation, characterized by significantly reduced bacterial richness ($p \leq 0.005$; number of bacterial types detected; Figure 2A), evenness ($p \leq 0.04$; relative

distribution of bacterial types; Figure 2B) and diversity ($p \leq 0.01$; metric calculated using richness and evenness indices; Figure 2C).

[0094] Reduced diversity was further demonstrated by hierarchical cluster analysis which revealed that the majority of healthy subjects clustered in a single tightly-knit group, with bacterial communities compositionally distinct from CRS patients who clustered into two distinct groups (Figure 2D). Healthy subject CRS 14, clustered with CRS patients, and it was subsequently determined that this individual had historically suffered from chronic nasal allergies. The present analysis thus confirms that the sinus microbiota composition of healthy subjects is distinct from that of CRS patients and, that within the CRS population, discrete sub-groups with distinct microbial community profiles exist.

[0095] Antibiotic use was examined, as it influences microbiota composition. All study subjects (CRS and healthy) received prophylactic antibiotics immediately (1 hour) prior to surgery and sample collection according to standard practice. Long-term pre-operative antibiotic use was absent for the majority of healthy subjects, and variable in the CRS patient group (Table 1). Patients with disparate long-term antimicrobial administration histories (*e.g.* CRS 4, 6 and 2; Group II; Table 1) clustered closely together. Moreover, Group II included two patients who had not received long-term antibiotics, and who exhibited significantly lower ($p < 0.006$) sinus community diversity compared to microbiota from patients in Group I. Group I subjects had received long-term prophylactic antibiotic administration, indicating that while antibiotic therapy may contribute, it is not the sole selective pressure defining bacterial community composition and loss of diversity in this niche.

C. Example 2: Taxa characteristic of healthy and CRS sinuses

[0096] Results from the Sino-Nasal Outcomes Test (SNOT-20) questionnaire, a metric to score sinus symptomology, confirmed that CRS patients reported significantly higher scores (*i.e.*, more severe sinus symptomology ($p \leq 0.003$)) than healthy controls. The observed microbiota clustering patterns were consistent with patient-reported sinus symptomology, as shown in Figure 2D). Given this level of independent validation of disease activity, specific community members that differentiated healthy subjects and CRS patients were next characterized at the taxonomic level.

[0097] Known pathogenic members of the Pseudomonadaceae, Lachnospiraceae, Ralstoniaceae, Mycobacteriaceae and Helicobacteriaceae were detected in both CRS patients with, and healthy subjects without, sinonasal symptoms. Thus, the mere detection of a suspected or known pathogen in a given niche does not necessarily imply pathogenic activity,

indicating that the microbiota composition at a given site may play a large role in defining the activity of community members.

[0098] Following correction for false discovery ($p \leq 0.05$, $q \leq 0.05$), a total of 1,482 taxa were detected in significantly lower relative abundance in CRS patient sinuses, underscoring the extent of sinus microbiota collapse in the CRS patient population. A large number of taxa exhibiting the most significant reductions in relative abundance in the CRS patients belonged to the order Lactobacillales and included probiotic species such as *Lactobacillus sakei*, as well as other phylogenetically distinct lactic acid bacteria (LAB) such as *Carnobacterium alterfunditum, Enterococcus mundtii* and *Pediococcus pentosaceus* implicating LAB in maintenance of healthy sinus mucosa. In stark comparison, only Corynebacteria, in particular, *Corynebacterium tuberculostearicum*, (Taxon ID 1493, str. CIP102346) exhibited a significant increase in abundance in CRS patients (Correlation value: 0.6220027; $p \leq 0.03$, $q \leq 0.003$). *Corynebacterium segmentosum* (Taxon ID 1192, str. CIP107068 (CCUG37878)) was also correlated with CRS (Correlation value: 0.5514262; $p \leq 0.05$).

[0099] The microbiota data were examined to identify those species that correlated with SNOT-20 symptom severity scores, in order to further confirm the clinical significance of these findings. A large group of 228 taxa were significantly ($p < 0.05$) correlated with lower SNOT-20 scores (indicative of healthy sinuses; Figure 3; Table X). Amongst these taxa were members of the LAB, *e.g.*, Lactobacilliaceae, Enterococcaceae, Aerococcaceae, and Streptococcaceae, further supporting the idea that these families are protective in healthy sinuses.

[0100] As noted above, the relative abundance of only two bacterial species was positively correlated with increased symptom severity; both belonged to the Corynebacteriaceae, with *C. tuberculostearicum* most correlated with symptom severity ($r = 0.62$; $p \leq 0.02$). Q-PCR analysis was performed using primers designed to specifically amplify *C. tuberculostearicum* to validate these findings. Linear regression of *C. tuberculostearicum* Q-PCR-derived copy number against both array-reported fluorescence intensity or against SNOT-20 score demonstrated concordance ($r = 0.66$; $p \leq 0.01$ and $r = 0.68$; $p \leq 0.01$ respectively). The regression data thus corroborate the array-based findings and confirm a strong relationship between the abundance of this species and sinus symptom severity (Figure 4).

D. Example 3: Murine model of sinusitis

[0101] To determine whether *C. tuberculostearicum*, which is typically considered a skin commensal, exhibited any pathogenic potential, a murine model of sinus infection was

developed. Goblet cell hyperplasia and mucin hypersecretion were used as indicators of pathology (*12*), and as the outcome measures to define pathogenic activity in the sinuses. This model is also allows for determination of whether *C. tuberculostearicum* is influenced by resident microbiota in the sinus cavity.

[0102] Four groups of mice (n = 5 animals per group) representing: i. Untreated control; ii. Antibiotic treated (to elicit microbiome depletion); iii. *C. tuberculostearicum* (ATCC #35694) inoculated; and iv. Antibiotic treated and *C. tuberculostearicum* inoculated animals were used (Figure 5). Q-PCR analyses of total 16S rRNA copy number from the sinuses of these animals confirmed that the burden of bacteria in the antibiotic treated groups was significantly lower ($p > 0.03$) than that of untreated animals. The data confirm acute antimicrobial depletion of bacterial burden and mucosal sinus microbiota diversity in antibiotic-treated groups. Histological examination of the sinus mucosa from each group demonstrated that the untreated control and antibiotic treated animals did not exhibit aberrant epithelial physiology (Figure 6Ai and ii), while instillation of *C. tuberculostearicum* in the presence of a replete sinus microbiota elicited a modest increase in the number of mucin-secreting goblet cells (Figure 6Aiii).

[0103] Animals treated with both antimicrobial and *C. tuberculostearicum* exhibited profound goblet cell hyperplasia, (Figure 6Aiv; Figure 6B and Table 2), significantly greater than that observed in any other group. Since mucin hypersecretion is a hallmark of respiratory infection (*18*) and chronic sinusitis (*12, 13*), these data confirm that *C. tuberculostearicum* is capable of inducing a characteristic response to pathogenic microbes and that this response is significantly augmented under conditions of depleted sinus microbiota.

Table 2.p-values for goblet cell number per μm of epithelial cell surface compared across mouse treatment groups (Figure 6).

	AB- CT-	AB+ CT-	AB- CT+	AB+ CT+
AB- CT-	-	0.36	0.011	0.0034
AB+ CT-	-	-	0.0012	0.0001
AB- CT+	-	-	-	0.0076
AB+ CT+	-	-	-	-

[0104] The above experiment was repeated, with inclusion of an additional group treated with antibiotics prior to instillation of *L. sakei* (ATCC 15521) (Figure 5), to demonstrate that goblet cell hyperplasia and mucin hypersecretion were induced specifically by *C. tuberculostearicum*. The conditions were designed to confirm that the characteristic pathogenic response did not simply represent a host response to instillation of any bacterial

species into the sinus niche. *L. sakei* was selected because this species was present in high abundance in healthy mucosal samples, and was the most significantly depleted taxon in CRS patients. These results indicated that *L. sakei* is a protective sinus mucosal colonizer, and likely would not induce a pathogenic response.

[0105] Q-PCR was performed on all treatment groups to confirm that animals receiving bacterial inocula exhibited the presence of these species (Figure 7). Histological imaging of the maxillary sinuses (Figure 8A) demonstrated again that the antibiotic-treated and *C. tuberculostearicum* inoculated group exhibited significant increases in goblet cell hyperplasia and mucin hypersecretion (Figure 8Aiii). Mice receiving identical numbers of *L. sakei*, however, demonstrated epithelial physiology comparable to that of control animals (no significant differences in goblet cell numbers; Table 3; Figure 8Aiv). That *L. sakei* inoculated animals did not display the pathogenic response indicated that it was specifically due to *C. tuberculostearium*. Enumeration of goblet cell numbers in each treatment group confirmed these observations (Figure 8B; Table 3).

[0106] The collective data indicate that *L. sakei* is protective in the sinus mucosa. We next investigated whether co-instillation of *L. sakei* with *C. tuberculostearicum* could abrogate the goblet cell hyperplasia and mucin hypersecretion phenotype induced by *C. tuberculostearicum*, even in the context of a depleted native microbiota. Following treatment with antibiotics, equal numbers of both species were instilled into the sinuses of mice. Histological examination revealed sinus epithelia comparable to that of animals in the control groups (Figure 8Av), with no significant differences in goblet cell numbers observed across these groups (Figure 8B and Table 3). Q-PCR analyses demonstrated significantly ($p < 0.02$) reduced *C. tuberculostearicum* abundance in the co-instilled animals compared to animals infected with *C. tuberculostearicum* alone (Figure 7). *L. sakei* numbers in these animals were, however, similar to those in animals treated with *L. sakei* alone (Figure 8). The data indicate that *L. sakei* protects the sinus epithelium, putatively through competitive inhibition of *C. tuberculostearicum*. *L. sakei* thus represents a novel, probiotic therapy for amelioration or prevention of sinus pathology, even in patients with severe sinus microbiome depletion.

Table 3. Significance values for comparative goblet cell number per μm of epithelial cell surface across mouse treatment groups (Figure 8).

	AB- CT-	AB+ CT-	AB+ LS+	AB+ CT+	AB+ CT+ LS+
AB- CT-	-	1	0.2002	0.0099	0.2082
AB+ CT-	-	-	0.2002	0.0099	0.2082
AB+ LS+	-	-	-	0.0118	0.5717
AB+ CT+	-	-	-	-	0.0141

AB+ CT+ LS+ - - - - -

Table X – Bacterial species negatively correlated with sinus symptom severity

Phylum	Family	Taxon	prokMSA Name	Estimate	P value	Q value
Firmicutes	Lactobacillaceae	3547	Lactobacillus frumenti str. TMW 1.666	-0.5324516	0.04998019	0.035270589
Proteobacteria	Helicobacteraceae	10520	Helicobacter sp. blood isolate 964	-0.5325229	0.04994373	0.035270589
Proteobacteria	Campylobacteraceae	10538	Arcobacter cryaerophilus	-0.5332394	0.04957863	0.035270589
Unclassified	Unclassified	243	hot spring clone OPB25	-0.5332699	0.04956314	0.035270589
Proteobacteria	Nitrospinaceae	594	uranium mining mill tailing clone GR-296.II.52 GR-296.I.52	-0.5334925	0.04945017	0.035270589
Firmicutes	Acholeplasmataceae	3977	Chinaberry yellows phytoplasma str. CbY1	-0.53364	0.04937536	0.035270589
Bacteroidetes	Porphyromonadaceae	5429		-0.5336558	0.04936737	0.035270589
Cyanobacteria	Chloroplasts	5040	Solanum nigrum	-0.5336963	0.04934686	0.035270589
Proteobacteria	Bradyrhizobiaceae	7390	Afipia genosp. 10 str. G8996	-0.5336971	0.04934647	0.035270589
Firmicutes	Lachnospiraceae	2994	termite gut clone Rs-L15	-0.533732	0.04932878	0.035270589
Synergistes	Unclassified	117	termite gut homogenate clone Rs-D89	-0.5342366	0.04907378	0.035270589
Bacteroidetes	Flavobacteriaceae	5401	Capnocytophaga gingivalis str. ChDC OS45	-0.5343746	0.04900422	0.035270589
Proteobacteria	Desulfobacteraceae	10046	Desulfobacterium cetonicum str. DSM 7267 oil recovery water	-0.5343791	0.04900192	0.035270589
Verrucomicrobia	Verrucomicrobiaceae	613	hydrothermal vent polychaete mucous clone P. palm C 85	-0.5344217	0.04898049	0.035270589
Proteobacteria	Unclassified	10427	hydrothermal vent 9 degrees North East Rise Pacific Ocean clone CH3_17_BAC_16SrRNA_9 N_EPR	-0.5344767	0.04895279	0.035270589
Actinobacteria	Micrococcaceae	1686	Yania halotolerans str. YIM 70085	-0.5345007	0.04894071	0.035270589
Unclassified	Unclassified	4410		-0.5345541	0.04891381	0.035270589
Cyanobacteria	Unclassified	5010	LPP-group cyanobacterium isolate str. QSSC5cya QSSC5cya	-0.5346143	0.04888353	0.035270589
Acidobacteria	Unclassified	6357	anaerobic benzene-degrading clone Cart-N4	-0.5346545	0.04886331	0.035270589
Proteobacteria	Unclassified	8961	Calyptogena magnifica symbiont	-0.5349561	0.04871181	0.035270589
Firmicutes	Acholeplasmataceae	3945	Ash witches'-broom phytoplasma str. AshWB	-0.5352908	0.04854413	0.035270589
Firmicutes	Lachnospiraceae	2693	ruminantium str. GA195	-0.5353908	0.04849409	0.035270589
Firmicutes	Peptococc/ Acidaminococc	865	Selenomonas sputigena str. ATCC 35185	-0.5354898	0.04844462	0.035270589
Firmicutes	Paenibacillaceae	3641	Brevibacillus sp. MN 47.2a aerobic basin clone	-0.5355341	0.04842245	0.035270589
Planctomycetes	Planctomycetaceae	4855	CY0ARA026D10	-0.5360602	0.0481602	0.035270589
Firmicutes	Lactobacillaceae	3696	Lactobacillus kalixensis str. Kx127A2; LMG 22115T; DSM 16043T; CCUG 48459T	-0.536599	0.04789264	0.035270589
Proteobacteria	Unclassified	10259		-0.5369322	0.04772772	0.035270589
Firmicutes	Unclassified	2541	UASB granular sludge clone	-0.5369672	0.04771044	0.035270589

			UT-2 termite gut homogenate			
Firmicutes	Lachnospiraceae	2681	clone Rs-K41 bacterium	-0.5370585	0.04766533	0.035270589
			ground water deep-well injection disposal site radioactive wastes Tomsk-7 clone S15A-MN96			
Proteobacteria	Bradyrhizobiaceae	7126	proteobacterium	-0.5371332	0.04762847	0.035270589
			trichloroethene-contaminated site clone FTLM142			
Firmicutes	Syntrophomonadaceae	2483	bacterium	-0.5376491	0.04737434	0.035270589
Firmicutes	Peptococc/ Acidaminococc	131	pig feces clone	-0.5377144	0.04734226	0.035270589
			Mars Odyssey Orbiter and encapsulation facility clone			
Proteobacteria	Unclassified	8587	T5-3	-0.5377735	0.04731322	0.035270589
			Capnocytophaga sputigena			
Bacteroidetes	Flavobacteriaceae	5906	str. ATCC 33612	-0.5378696	0.04726602	0.035270589
			Flavobacterium columnare			
Bacteroidetes	Flavobacteriaceae	5933	str. PH-97028 (IAM 14821)	-0.5378938	0.04725415	0.035270589
			oral endodontic infection			
Firmicutes	Lachnospiraceae	2965	clone MCE9_173	-0.5381045	0.04715086	0.035270589
			Arthrobacter agilis str. DSM			
Actinobacteria	Micrococcaceae	1494	20550	-0.5383154	0.04704757	0.035270589
BRC1	Unclassified	5143	soil clone PBS-II-1	-0.5383959	0.04700824	0.035270589
			Enterococcus saccharolyticus			
Firmicutes	Enterococcaceae	3298	str. LMG 11427	-0.5391099	0.04666012	0.035270589
Cyanobacteria	Unclassified	5038	Rumen isolate str. YS2	-0.5392866	0.04657427	0.035270589
			termite gut homogenate			
Firmicutes	Lachnospiraceae	4335	clone Rs-N86 bacterium	-0.5392971	0.04656915	0.035270589
			Marinilactibacillus			
Firmicutes	Aerococcaceae	3504	psychrotolerans str. O21	-0.5397087	0.04636966	0.035270589
Bacteroidetes	Unclassified	5257	marine? clone KD3-67	-0.5398265	0.04631263	0.035270589
			chlorobenzene-degrading			
Firmicutes	Peptococc/ Acidaminococc	534	consortium clone IIA-26	-0.5401839	0.04614008	0.035270589
Spirochaetes	Spirochaetaceae	6526	Treponema sp. str. 7CPL208	-0.5402284	0.0461186	0.035270589
			Arthrobacter nicotianae str.			
Actinobacteria	Micrococcaceae	1573	SB42	-0.5405694	0.04595441	0.035270589
Proteobacteria	Desulfoarculaceae	10227	marine sediment clone Boll1	-0.5409671	0.04576348	0.035270589
Firmicutes	Clostridiaceae	4278	granular sludge clone R1p16	-0.5409777	0.04575841	0.035270589
Proteobacteria	Unclassified	7060		-0.5410353	0.0457308	0.035270589
			Mono Lake at depth 35 m station 6 July 2000 clone			
Bacteroidetes	Unclassified	5785	ML635J-56	-0.5413643	0.04557336	0.035270589
			Streptococcus constellatus			
Firmicutes	Streptococcaceae	3499	str. ATCC27823	-0.5416222	0.04545022	0.035270589
			Clostridium subterminale			
Firmicutes	Clostridiaceae	4177	DSM 2636	-0.5419209	0.04530792	0.035270589
			termite gut homogenate			
Bacteroidetes	Rikenellaceae	5889	clone Rs-F73 bacterium	-0.5420021	0.0452693	0.035270589
			uranium mining waste pile clone JG37-AG-139			
Proteobacteria	Bdellovibrionaceae	10010	proteobacterium	-0.5422324	0.04515985	0.035270589
			oral endodontic infection			
Firmicutes	Peptococc/ Acidaminococc	392	clone MCE7_134	-0.5424949	0.04503537	0.035270589
			crevicular epithelial cells			
Bacteroidetes	Sphingobacteriaceae	5513	clone AZ123	-0.5425299	0.04501877	0.035270589
			Mycoplasma			
Firmicutes	Mycoplasmataceae	3929	gypsbengalensis str. Gb-V33	-0.5427064	0.04493525	0.035270589
Firmicutes	Clostridiaceae	4300	termite gut clone Rs-060	-0.5428522	0.04486631	0.035270589

Proteobacteria	Campylobacteraceae	10484	Campylobacter helveticus	-0.5430959	0.04475126	0.035270589
OP10	Unclassified	514	sludge clone SBRA136	-0.5432395	0.04468356	0.035270589
Proteobacteria	Syntrophobacteraceae	10221	granular sludge clone R3p4	-0.5433823	0.04461634	0.035270589
Firmicutes	Lactobacillaceae	3330	Lactobacillus kitasatonis str. KM9212	-0.5437728	0.04443286	0.035270589
BRC1	Unclassified	118	penguin droppings sediments clone KD1-1	-0.5440147	0.04431948	0.035270589
Firmicutes	Peptococc/ Acidaminococc	39	forested wetland clone RCP2-71	-0.5441698	0.04424689	0.035270589
Proteobacteria	Pasteurellaceae	9213	Haemophilus quentini str. MCCM 02026	-0.544316	0.04417854	0.035270589
Firmicutes	Streptococcaceae	3629	Streptococcus mutans str. UA96	-0.5443712	0.04415277	0.035270589
Proteobacteria	Unclassified	9760	deep marine sediment clone MB-A2-137	-0.5450115	0.0438545	0.035270589
Firmicutes	Unclassified	926		-0.5457011	0.04353492	0.035270589
Firmicutes	Staphylococcaceae	3524	Gemella haemolysans	-0.5458225	0.04347884	0.035270589
Proteobacteria	Syntrophobacteraceae	10021	uranium mill tailings soil sample clone Sh765B-TzT-29 proteobacterium	-0.546055	0.04337153	0.035270589
Actinobacteria	Micrococcaceae	1593	Arthrobacter globiformis	-0.5462532	0.04328029	0.035270589
Proteobacteria	Desulfobacteraceae	9875	hydrothermal sediment clone AF420354	-0.5463408	0.04323997	0.035270589
Cyanobacteria	Chloroplasts	4966	Adiantum pedatum	-0.5470294	0.04292415	0.035270589
Bacteroidetes	Prevotellaceae	5893	tongue dorsa clone DO045	-0.5471467	0.04287049	0.035270589
Firmicutes	Peptococc/Acidaminococc	562	oral endodontic infection clone MCE10_265	-0.5472263	0.04283412	0.035270589
Chlorobi	Unclassified	636	benzene-degrading nitrate-reducing consortium clone Cart-N3 bacterium	-0.5473972	0.04275611	0.035270589
Firmicutes	Carnobacteriaceae	3536		-0.5475614	0.04268128	0.035270589
Bacteroidetes	Flavobacteriaceae	5473		-0.5478064	0.04256979	0.035270589
Proteobacteria	Syntrophobacteraceae	9845	uranium mining waste pile clone JG37-AG-128 proteobacterium	-0.5484553	0.0422755	0.035270589
Proteobacteria	Campylobacteraceae	10540	Campylobacter showae str. LMG 12636	-0.5489328	0.0420599	0.035270589
Proteobacteria	Sphingomonadaceae	7100	Novosphingobium capsulatum str. GIFU11526	-0.5490852	0.04199126	0.035270589
Proteobacteria	Bradyrhizobiaceae	6887	Bradyrhizobium str. YB2	-0.5492974	0.04189579	0.035270589
Chloroflexi	Unclassified	76	DCP-dechlorinating consortium clone SHA-147	-0.5500398	0.04156317	0.035270589
Firmicutes	Clostridiaceae	4265	termite gut homogenate clone Rs-N70 bacterium	-0.5500563	0.04155579	0.035270589
Bacteroidetes	Flavobacteriaceae	5436	Arctic sea ice ARK10004	-0.5501646	0.04150741	0.035270589
Actinobacteria	Unclassified	1898	termite gut homogenate clone Rs-J10 bacterium	-0.5503548	0.0414226	0.035270589
Firmicutes	Aerococcaceae	3631	Abiotrophia defectiva str. GIFU12707 (ATCC49176)	-0.5504543	0.04137826	0.035270589
Firmicutes	Streptococcaceae	3753	Streptococcus suis str. 8074	-0.5505636	0.04132961	0.035270589
Bacteroidetes	Flexibacteraceae	6124	Flexibacter flexilis subsp. pelliculosus str. IFO 16028 subsp.	-0.5507177	0.04126111	0.035270589
Firmicutes	Enterococcaceae	3382		-0.5508457	0.04120423	0.035270589
Firmicutes	Leuconostocaceae	3573	Leuconostoc ficulneum str. FS-1	-0.5511595	0.0410651	0.035270589
Firmicutes	Streptococcaceae	3422	Streptococcus thermophilus str. DSM 20617	-0.5511839	0.04105433	0.035270589
Firmicutes	Lachnospiraceae	4315	termite gut homogenate	-0.5512027	0.04104598	0.035270589

Unclassified	Unclassified	7444	clone Rs-N94 bacterium	-0.5513268	0.04099107	0.035270589
Bacteroidetes	Flexibacteraceae	6261	Arctic sea ice cryoconite clone ARKCRY-50	-0.5515557	0.04088996	0.035270589
Planctomycetes	Planctomycetaceae	4948	anoxic basin clone CY0ARA027D01	-0.5524994	0.0404749	0.035270589
Firmicutes	Streptococcaceae	3253	derived cheese sample clone 32CR	-0.5527952	0.04034546	0.035270589
Firmicutes	Clostridiaceae	4614	Clostridium sp. str. JC3	-0.5534251	0.04007081	0.035270589
Bacteroidetes	Flavobacteriaceae	5267	bacterioplankton clone AEGEAN_179	-0.5535169	0.04003091	0.035270589
Natronoanaerobium	Unclassified	4377	Mono Lake at depth 35m station 6 July 2000 clone ML635J-65 G+C	-0.5538773	0.03987448	0.035270589
OP9/JS1	Unclassified	2489	Guaymas Basin hydrothermal vent sediments clone B01R005	-0.5543007	0.0396913	0.035270589
Chloroflexi	Unclassified	2534	forest soil clone S085	-0.5543422	0.03967338	0.035270589
Bacteroidetes	Unclassified	5957	hydrothermal vent polychaete mucous clone P. palm C/A 20	-0.5543543	0.03966819	0.035270589
Firmicutes	Lactobacillaceae	3521	Pediococcus inopinatus str. DSM 20285	-0.555167	0.03931844	0.035270589
Firmicutes	Unclassified	77	thermal soil clone YNPFFP9	-0.5552835	0.03926852	0.035270589
Firmicutes	Clostridiaceae	4550	swine intestine clone p-320-a3	-0.5558022	0.0390467	0.035270589
Firmicutes	Lachnospiraceae	2668	termite gut homogenate clone Rs-G40 bacterium	-0.5558071	0.03904461	0.035270589
Actinobacteria	Micrococcaceae	1557	Arthrobacter oxydans str. DSM 20119	-0.5560342	0.03894779	0.035270589
Firmicutes	Clostridiaceae	4459	termite gut clone Rs-109	-0.5567823	0.03863011	0.035270589
Bacteroidetes	Flavobacteriaceae	5521	Flavobacteriaceae str. SW269	-0.5568853	0.03858655	0.035270589
Proteobacteria	Campylobacteraceae	10397	groundwater clone 1006	-0.5570026	0.03853694	0.035270589
Proteobacteria	Unclassified	9876	deep marine sediment clone MB-B2-106	-0.5570065	0.03853531	0.035270589
Firmicutes	Lachnospiraceae	4514	termite gut homogenate clone Rs-B34 bacterium	-0.5570651	0.03851051	0.035270589
Proteobacteria	Polyangiaceae	9733	bacterioplankton clone ZA3735c	-0.5574153	0.03836281	0.035270589
Firmicutes	Unclassified	522	UASB granular sludge clone UT-1	-0.5575107	0.03832264	0.035270589
Firmicutes	Streptococcaceae	3722	Lactococcus lI1403 subsp. lactis str. IL1403	-0.5578807	0.03816718	0.035270589
Firmicutes	Peptococc/ Acidaminococc	304	Selenomonas ruminantium str.JCM6582	-0.5580707	0.0380875	0.035270589
Firmicutes	Lactobacillaceae	3885	Pediococcus pentosaceus	-0.5582024	0.03803234	0.035270589
Proteobacteria	Sphingomonadaceae	7440	Sphingobium chungbukense str. DJ77	-0.558408	0.03794637	0.035270589
Firmicutes	Aerococcaceae	3326	Nostocoida limicola I str. Ben206	-0.558438	0.03793384	0.035270589
Bacteroidetes	Flexibacteraceae	5602		-0.558608	0.03786285	0.035270589
Proteobacteria	Polyangiaceae	10298	marine tidal mat clone BTM36	-0.5588222	0.03777357	0.035270589
Firmicutes	Bacillaceae	3900	Bacillus licheniformis str. DSM 13	-0.5588343	0.03776853	0.035270589
Bacteroidetes	Rikenellaceae	5892	anoxic bulk soil flooded rice microcosm clone BSV73	-0.559133	0.03764434	0.035270589
Proteobacteria	Pasteurellaceae	8195	Haemophilus influenzae str. R2866	-0.5591662	0.03763053	0.035270589

Chlorobi	Chlorobiaceae	859	Chlorobium phaeovibrioides str. 2631	-0.5598611	0.03734276	0.035270589
Firmicutes	Clostridiaceae	4477	termite gut homogenate clone Rs-N85 bacterium	-0.5599449	0.03730819	0.035270589
Proteobacteria	Unclassified	244	deep marine sediment clone MB-C2-152	-0.5600791	0.03725282	0.035270589
Bacteroidetes	Flavobacteriaceae	6248	Capnocytophaga sp. oral strain str. S3	-0.56046	0.03709607	0.035270589
Proteobacteria	Unclassified	10084	acid mine drainage clone AS6	-0.5606514	0.03701749	0.035270589
Firmicutes	Streptococcaceae	3588	Streptococcus downei str. ATCC 33748	-0.5606556	0.03701576	0.035270589
Firmicutes	Peptococc/ Acidaminococc	1036	Great Artesian Basin clone G07	-0.560657	0.0370152	0.035270589
Chloroflexi	Unclassified	2397	deep marine sediment clone MB-C2-127	-0.561111	0.0368293	0.035270589
Actinobacteria	Micrococcaceae	2020	Rothia dentocariosa str. ChDC B200	-0.5612027	0.03679183	0.035270589
Bacteroidetes	Unclassified	5353	trichloroethene-contaminated site clone FTLpost3 bacterium	-0.561455	0.03668889	0.035270589
Bacteroidetes	Flavobacteriaceae	5991	Tenacibaculum ovolyticum str. IAM14318	-0.5617321	0.03657605	0.035270589
Proteobacteria	Sphingomonadaceae	7036	Lutibacterium anuloederans str. LC8	-0.562055	0.03644492	0.035270589
Cyanobacteria	Chloroplasts	5147	Emiliania huxleyi str. Plymouth Marine Laborator PML 92	-0.5620697	0.03643899	0.035270589
Actinobacteria	Acidimicrobiaceae	1090		-0.5623333	0.03633219	0.035270589
Proteobacteria	Unclassified	9813	hydrothermal sediment clone AF420340	-0.5624152	0.03629906	0.035270589
Firmicutes	Enterococcaceae	3433	Tetragenococcus muriaticus	-0.5626691	0.03619651	0.035270589
Firmicutes	Acholeplasmataceae	4044		-0.5626807	0.03619181	0.035270589
TM7	Unclassified	8040	oral cavity clone BE109	-0.5630985	0.03602354	0.035270589
Bacteroidetes	Porphyromonadaceae	5295	swine intestine clone p-987-s962-5	-0.563282	0.03594978	0.035270589
Bacteroidetes	Flavobacteriaceae	5914	Psychroserpens burtonensis str. S2-64	-0.563714	0.03577667	0.035270589
Firmicutes	Lachnospiraceae	4613	rumen clone 3C0d-3	-0.5638647	0.03571638	0.035270589
Firmicutes	Acholeplasmataceae	4046	Pigeon pea witches'-broom mycoplasma-like organism	-0.56396	0.03567834	0.035270589
Bacteroidetes	Flavobacteriaceae	6269	acidic forest soil clone UC1	-0.5640842	0.03562876	0.035270589
OP9/JS1	Unclassified	969	DCP-dechlorinating consortium clone SHA-1	-0.5641236	0.03561305	0.035270589
Firmicutes	Clostridiaceae	4180	termite gut homogenate clone Rs-M23 bacterium	-0.5642019	0.03558187	0.035270589
Cyanobacteria	Chloroplasts	4976	Calypogeia muelleriana	-0.5642267	0.03557199	0.035270589
Firmicutes	Enterococcaceae	3261	Enterococcus mundtii str. LMG 10748	-0.5647034	0.03538246	0.035270589
Actinobacteria	Micrococcaceae	1324	glacial ice isolate str. CanDirty1	-0.5655964	0.03502939	0.035270589
Firmicutes	Peptococc/ Acidaminococc	761	Anaeroglobus geminatus str. AIP313.00; CIP 106856; CCUG 44773	-0.5657127	0.0349836	0.035270589
Bacteroidetes	Unclassified	5475	SHA-25 clone	-0.5660295	0.03485913	0.035270589
Planctomycetes	Planctomycetaceae	4831	Planctomyces brasiliensis	-0.566052	0.0348503	0.035270589
Firmicutes	Lachnospiraceae	4510	termite gut homogenate clone Rs-Q53 bacterium	-0.5663472	0.0347346	0.035270589
Firmicutes	Lactobacillaceae	3767	Lactobacillus suebicus str. CECT 5917T	-0.5665992	0.03463607	0.035270589

Firmicutes	Peptococc/ Acidaminococc	709	Selenomonas ruminantium str.S20	-0.5670817	0.03444798	0.035270589
Proteobacteria	Unclassified	9884	forested wetland clone RCP2-62	-0.5671464	0.03442282	0.035270589
Firmicutes	Leuconostocaceae	3497	Weissella koreensis S-5673	-0.5673939	0.03432668	0.035270589
Firmicutes	Enterococcaceae	3713	Enterococcus cecorum str. ATCC43198	-0.5677953	0.03417121	0.035270589
Firmicutes	Lactobacillaceae	3566	Lactobacillus pontis str. LTH 2587	-0.5690756	0.03367873	0.035270589
Firmicutes	Unclassified	4536	Mono Lake at depth 35m station 6 July 2000 clone ML635J-14 G+C	-0.5692171	0.03362463	0.035270589
Firmicutes	Unclassified	3481		-0.5692343	0.03361806	0.035270589
Proteobacteria	Syntrophobacteracea e	9661	DCP-dechlorinating consortium clone SHD-1	-0.5696648	0.0334539	0.035270589
Proteobacteria	Desulfobacteraceae	10364	marine surface sediment clone SB2	-0.5697152	0.03343469	0.035270589
Firmicutes	Lachnospiraceae	2943	human thigh wound isolate str. MDA2477	-0.5708144	0.03301837	0.035270589
Firmicutes	Enterococcaceae	3318	Enterococcus ratti str. ATCC 700914	-0.5711715	0.03288393	0.035270589
Bacteroidetes	Porphyromonadaceae	6012	mouse feces clone L11-6	-0.5712018	0.03287255	0.035270589
Firmicutes	Clostridiaceae	4554	termite gut clone Rs-068	-0.571372	0.03280865	0.035270589
Firmicutes	Lactobacillaceae	3490	Lactobacillus suntoryeus str. LH	-0.5714735	0.03277059	0.035270589
Firmicutes	Clostridiaceae	4582	swine intestine clone p-2600-9F5	-0.5725013	0.03238692	0.035270589
Firmicutes	Peptococc/ Acidaminococc	242	Desulfosporosinus orientis str. DSMZ 7493	-0.5726663	0.03232564	0.035270589
Firmicutes	Streptococcaceae	3907	aortic heart valve patient with endocarditis clone v6	-0.5727726	0.03228622	0.035270589
Firmicutes	Peptococc/ Acidaminococc	710	Centipeda periodontii str. HB-2	-0.5729265	0.03222922	0.035270589
Cyanobacteria	Chloroplasts	5183	Pisum sativum -- chloroplast	-0.5756529	0.03123133	0.035270589
Cyanobacteria	Chloroplasts	5192	Cycas revoluta	-0.5759699	0.03111681	0.035270589
Firmicutes	Lachnospiraceae	2991	rumen clone 3C3d-8	-0.5761139	0.03106487	0.035270589
Firmicutes	Enterococcaceae	3881	Enterococcus dispar str. LMG 13521	-0.5764427	0.03094657	0.035270589
Bacteroidetes	Flavobacteriaceae	5836	Capnocytophaga granulosa str. LMG 12119; FDC SD4	-0.5766249	0.03088116	0.035270589
Firmicutes	Peptococc/ Acidaminococc	300	benzene-contaminated groundwater clone ZZ12C8	-0.5787987	0.03010865	0.035270589
Bacteroidetes	Unclassified	5784	fruiting body Pleurotus eryngii clone PE01	-0.5792647	0.02994496	0.035270589
Firmicutes	Bacillaceae	3848	Bacillus sp. str. TUT1007	-0.5793954	0.02989915	0.035270589
Firmicutes	Carnobacteriaceae	3792	Carnobacterium sp. str. D35	-0.5804968	0.02951531	0.035270589
Firmicutes	Lachnospiraceae	4533	termite gut homogenate clone Rs-N06 bacterium	-0.581153	0.02928835	0.035270589
Unclassified	Unclassified	651		-0.5811737	0.02928121	0.035270589
Bacteroidetes	Flavobacteriaceae	6246	crevicular epithelial cells clone BU084	-0.5814157	0.02919784	0.035270589
Firmicutes	Lachnospiraceae	4434	termite gut homogenate clone Rs-K11 bacterium	-0.5814826	0.02917485	0.035270589
Firmicutes	Lactobacillaceae	3418	Lactobacillus subsp. aviarius	-0.5814892	0.02917259	0.035270589
Firmicutes	Clostridiaceae	4297		-0.5818952	0.02903323	0.035270589
Proteobacteria	Polyangiaceae	9671	hydrothermal sediment clone AF420357	-0.5821323	0.02895206	0.035270589
Firmicutes	Acholeplasmataceae	3976		-0.5828049	0.0287228	0.035270589
Firmicutes	Acholeplasmataceae	3961	Clover yellow edge mycoplasma-like organism	-0.5834912	0.02849025	0.035270589

Bacteroidetes	Flavobacteriaceae	5301		-0.5838861	0.02835706	0.035270589
Firmicutes	Streptococcaceae	3685	Streptococcus gordonii str. ATCC 10558	-0.5840426	0.02830442	0.035270589
Actinobacteria	Micrococcaceae	2063	Rothia dentocariosa str. ATCC 17931	-0.5849591	0.02799754	0.035270589
Firmicutes	Clostridiaceae	4272	termite gut homogenate clone Rs-M34 bacterium	-0.5856846	0.02775641	0.035270589
Synergistes	Unclassified	353	UASB reactor granular sludge clone PD-UASB-13 G+C	-0.5862744	0.02756153	0.035270589
Fusobacteria	Fusobacteriaceae	721	human mouth clone P2PB_51	-0.5863706	0.02752982	0.035270589
Firmicutes	Clostridiaceae	4524	termite gut clone Rs-093	-0.5872218	0.0272506	0.035270589
Firmicutes	Acholeplasmataceae	4045	Chinaberry yellows phytoplasma	-0.5874789	0.0271667	0.035270589
Firmicutes	Lachnospiraceae	4155	termite gut homogenate clone Rs-K92 bacterium	-0.5883278	0.02689095	0.035270589
Firmicutes	Lactobacillaceae	3703	Lactobacillus salivarius str. RA2115	-0.5896055	0.02647992	0.035270589
Firmicutes	Clostridiaceae	4622	termite gut clone Rs-L36	-0.590871	0.02607745	0.035270589
Firmicutes	Aerococcaceae	3833	Carnobacterium alterfunditum	-0.5911531	0.02598836	0.035270589
Firmicutes	Lachnospiraceae	4540	termite gut homogenate clone Rs-M18 bacterium	-0.5918608	0.02576588	0.035270589
Firmicutes	Streptococcaceae	3251	Streptococcus cristatus str. ATCC 51100	-0.5919206	0.02574714	0.035270589
TM7	Unclassified	2697	midgut homogenate Pachnoda ephippiata larva clone PeM47	-0.591935	0.02574265	0.035270589
Chloroflexi	Unclassified	258	DCP-dechlorinating consortium clone SHD-14	-0.5925901	0.02553809	0.035270589
Firmicutes	Aerococcaceae	3840	Trichococcus pasteurii str. KoTa2	-0.5926609	0.02551607	0.035270589
Proteobacteria	Neisseriaceae	8143	subgingival dental plaque clone AK105	-0.5935762	0.02523253	0.035270589
Firmicutes	Acholeplasmataceae	3975	Black raspberry witches'-broom phytoplasma str. BRWB witches'-broom	-0.5940016	0.02510154	0.035270589
Firmicutes	Streptococcaceae	3543		-0.5975365	0.02403278	0.035270589
Chloroflexi	Unclassified	2339	uranium mill tailings soil sample clone Sh765B-TzT-20 bacterium	-0.6019879	0.02273596	0.035270589
Firmicutes	Unclassified	3289	Isobaculum melis CCUG 37660T	-0.6020217	0.02272631	0.035270589
Firmicutes	Clostridiaceae	4475	termite gut homogenate clone Rs-N02 bacterium	-0.6038353	0.02221351	0.035270589
Firmicutes	Enterococcaceae	3598	Enterococcus solitarius str. DSM 5634	-0.6071271	0.02130506	0.035270589
Firmicutes	Clostridiaceae	4310	termite gut clone Rs-056	-0.6072521	0.02127112	0.035270589
Firmicutes	Enterococcaceae	3680	Melissococcus plutonius str. NCDO 2440	-0.609524	0.02066141	0.035270589
Firmicutes	Peptococc/ Acidaminococc	150		-0.6166294	0.01883948	0.035270589
Chlorobi	Unclassified	549	benzene-degrading nitrate-reducing consortium clone Cart-N2 bacterium	-0.6183396	0.01841973	0.035270589
Firmicutes	Lactobacillaceae	3526	Lactobacillus sakei	-0.6198186	0.01806246	0.035270589
Firmicutes	Lachnospiraceae	4281	granular sludge clone UASB_brew_B86	-0.6271224	0.01637464	0.035270589
Bacteroidetes	Flavobacteriaceae	5726	Bergeyella sp. oral AK152	-0.6281038	0.01615736	0.035270589

			clone			
			granular sludge clone			
Firmicutes	Lachnospiraceae	4512	UASB_brew_B25	-0.6320073	0.01531468	0.035270589
			granular sludge clone			
Firmicutes	Lachnospiraceae	4331	UASB_brew_B84	-0.6379284	0.01410088	0.035270589
			Lactobacillus frumenti str.			
Firmicutes	Lactobacillaceae	3547	TMW 1.666	-0.5324516	0.04998019	0.035270589

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WHAT IS CLAIMED IS:

1. A method of treating sinusitis in an individual in need thereof comprising:

administering at least one bacterial species listed in Table X to the individual, wherein at least one of the bacterial species is a Lactic Acid Bacterial (LAB) species;

allowing the bacterial species to colonize sinonasal mucosa of the individual, thereby treating sinusitis in the individual.

2. The method of claim 1, wherein at least five bacterial species listed in Table X are administered.

3. The method of claim 1 or 2, wherein Lactobacillus sakei is administered.

4. The method of claim 1, wherein one bacterial species is administered, and the bacterial species is Lactobacillus sakei.

5. The method of any one of the foregoing claims, wherein the colonized sinonasal mucosa is the maxillary sinus.

6. The method of any one of the foregoing claims, wherein the at least one bacterial species is administered nasally or to the sinuses.

7. The method of any one of the foregoing claims, further comprising detecting the microbial diversity of the sinus mucosa of the individual.

8. The method of any one of the foregoing claims, further comprising detecting the relative level of Corynebacterium in a mucosal sample from the individual, and

comparing the relative level of Corynebacterium in the sample to a control of Corynebacterium levels.

9. A pharmaceutical composition comprising at least one bacterial species listed in Table X, wherein at least one of the bacterial species is a Lactic Acid Bacterial (LAB) species, and a pharmaceutically acceptable excipient suitable for nasal administration.

10. The pharmaceutical composition of claim 9, comprising at least five bacterial species listed in Table X.

11. The pharmaceutical composition of claim 9, comprising Lactobacillus sakei.

12. The pharmaceutical composition of claim 9, consisting essentially of one bacterial species, wherein the bacterial species is Lactobacillus sakei.

13. A method of improving the sinus microbiota in an individual comprising:

administering at least one bacterial species listed in Table X to the individual, wherein at least one of the bacterial species is a Lactic Acid Bacterial (LAB) species;

allowing the bacterial species to colonize sinonasal mucosa of the individual, thereby improving the sinus microbiota in the individual.

14. The method of claim 13, wherein at least five bacterial species listed in Table X are administered.

15. The method of any one of claims 13- 15, wherein Lactobacillus sakei is administered.

16. The method of claim 13, wherein one bacterial species is administered, and the bacterial species is Lactobacillus sakei.

17. The method of any one of claims 13-16, wherein the colonized sinonasal mucosa is the maxillary sinus.

18. The method of any one of claims 13-17, further comprising detecting the microbial diversity of the sinonasal mucosa of the individual.

19. The method of any one of claims 13-18, further comprising detecting the relative level of Corynebacterium in a mucosal sample from the individual, and

comparing the relative level of Corynebacterium in the sample to a control of Corynebacterium levels.

20. A method of determining whether an individual has or is at risk of developing sinusitis, comprising:

detecting the relative level of Corynebacteria in a mucosal sample from the individual,

comparing the relative level of Corynebacteria in the sample to a control of Corynebacteria levels, and

determining that the individual has or is at risk of developing sinusitis where the relative level of Corynebacteria in the sample is

(i) higher than the control, wherein the control is a non-sinusitis control or

(ii) in the range of the control, wherein the control is a sinusitis-positive control.

21. The method of claim 20, wherein the Corynebacteria is Corynebacterium tuberculostearicum.

22. The method of claim 20 or 21, wherein the mucosal sample is from the maxillary sinus of the individual.

23. The method of any one of claims 20-22, comprising obtaining a mucosal sample from the maxillary sinus of the individual before detecting.

24. The method of any one of claims 20-23, further comprising administering a sinusitis therapeutic agent to the individual.

25. The method of claim 24, wherein the sinusitis therapeutic is a pharmaceutical composition comprising at least one bacterial species listed in Table X, wherein at least one of the bacterial species is a Lactic Acid Bacterial (LAB) species.

26. The method of claim 25, wherein the sinusitis therapeutic is a pharmaceutical composition comprising at least five bacterial species listed in Table X.

27. The method of claim 25 or 26, wherein the pharmaceutical composition comprises Lactobacillus sakei.

FIGURE 1

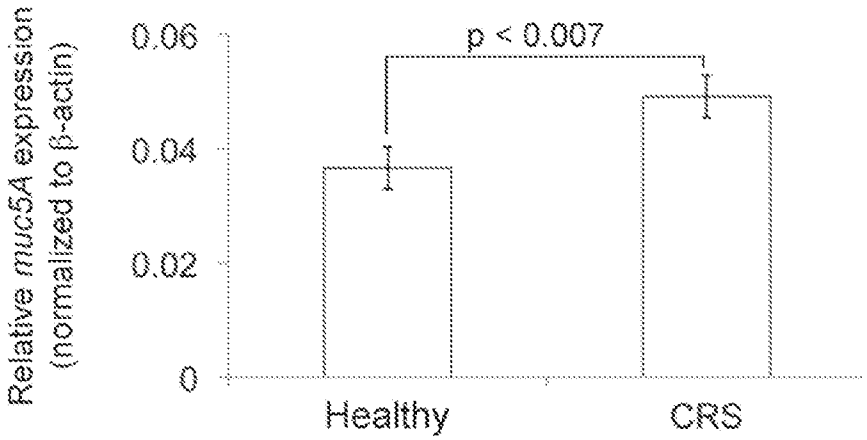

Relative muc5A expression
(normalized to β-actin)
0.06
0.04
0.02
0
p < 0.007
Healthy
CRS

FIGURE 2

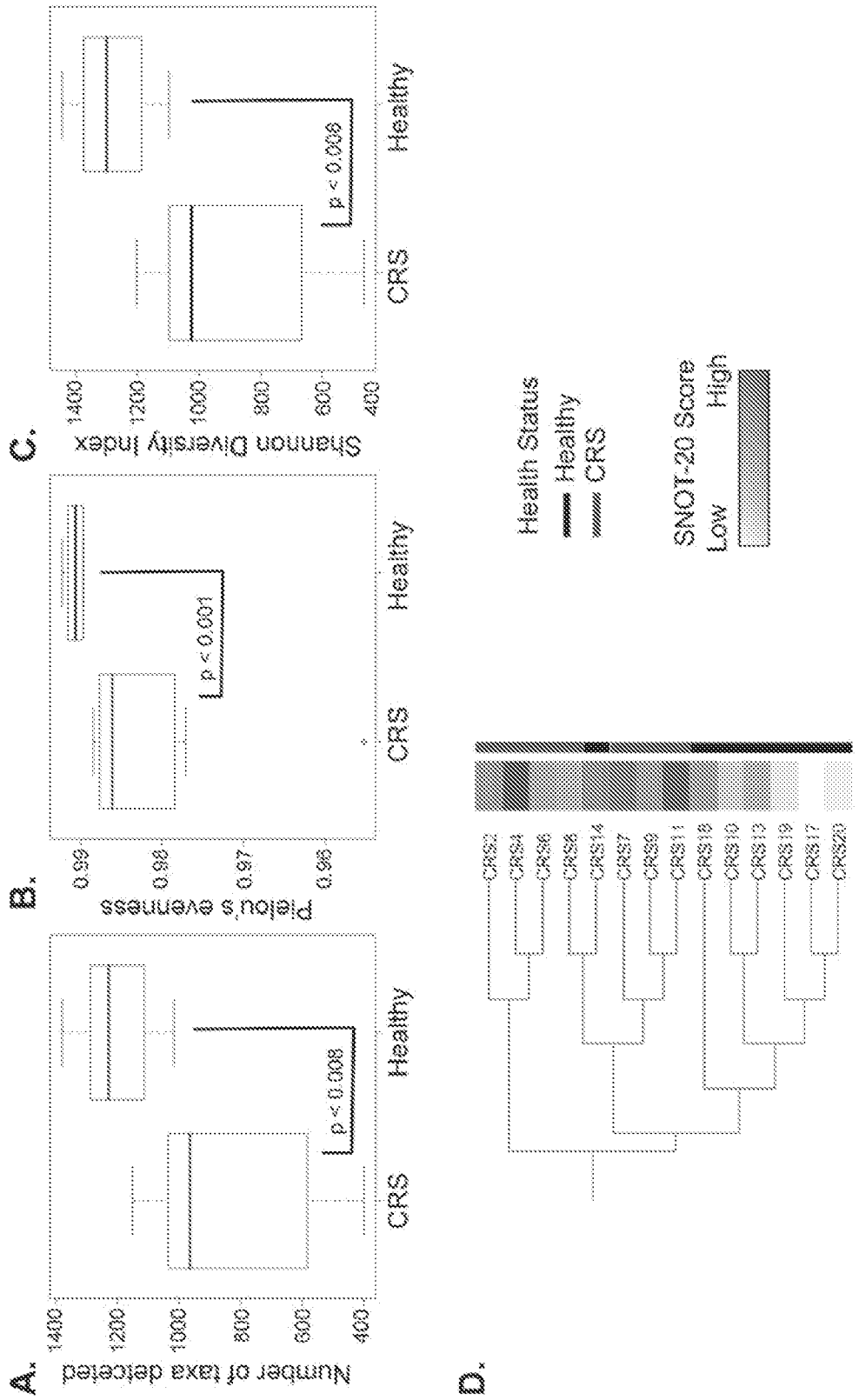

A.
Number of taxa detected
1400
1200
1000
800
600
400
p < 0.008
CRS
Healthy
B.
Pielou's evenness
0.99
0.98
0.97
0.96
p < 0.001
CRS
Healthy
C.
Shannon Diversity Index
1400
1200
1000
800
600
400
p < 0.006
CRS
Healthy
D.
Health Status
Healthy
CRS
SNOT-20 Score
Low
High
CRS2
CRS4
CRS5
CRS6
CRS14
CRS7
CRS9
CRS11
CRS18
CRS10
CRS13
CRS19
CRS17
CRS20

FIGURE 3

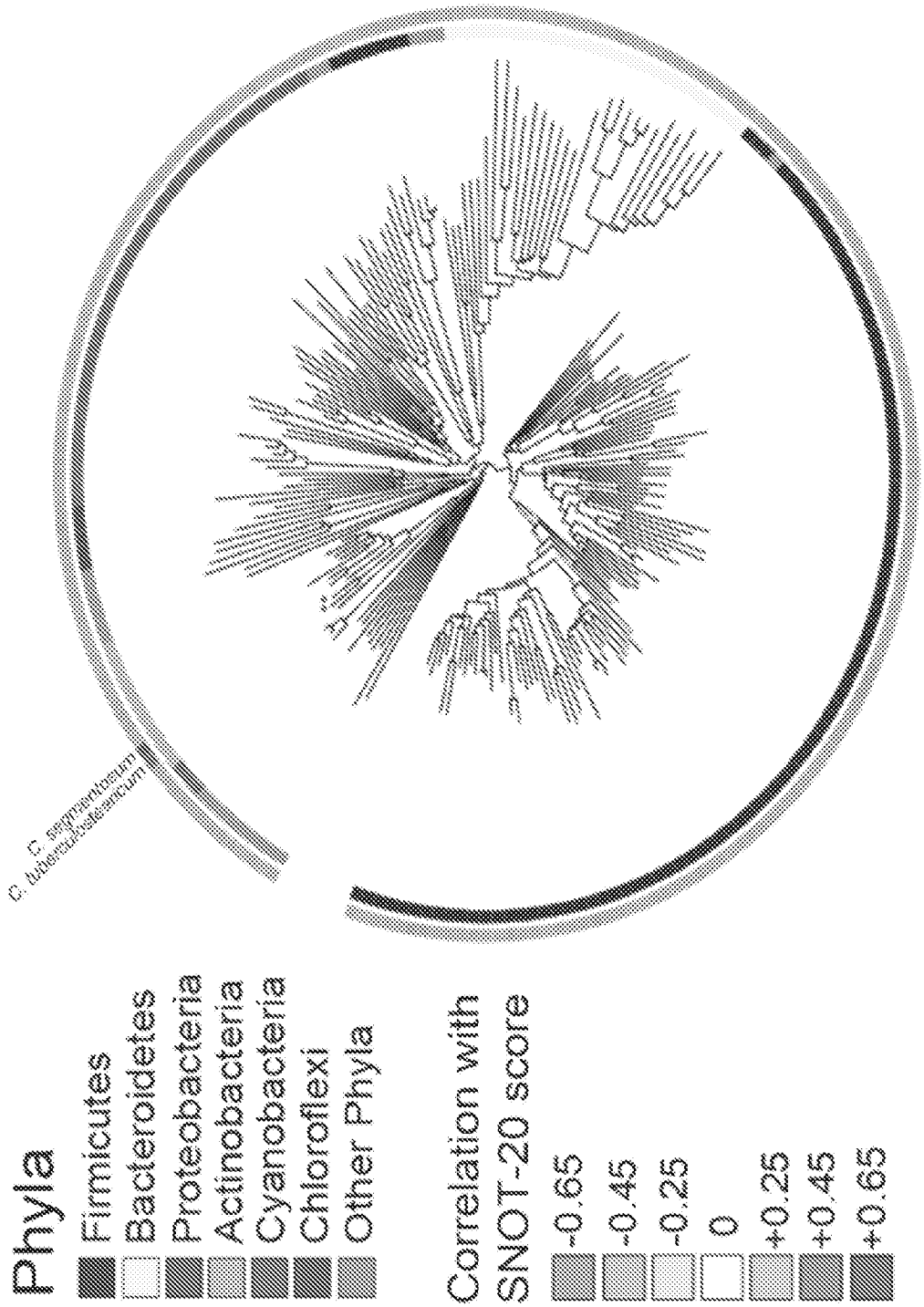

C. segmentosum
C. tuberculostearicum
Phyla
Firmicutes
Bacteroidetes
Proteobacteria
Actinobacteria
Cyanobacteria
Chloroflexi
Other Phyla
Correlation with SNOT-20 score
-0.65
-0.45
-0.25
0
+0.25
+0.45
+0.65

FIGURE 4

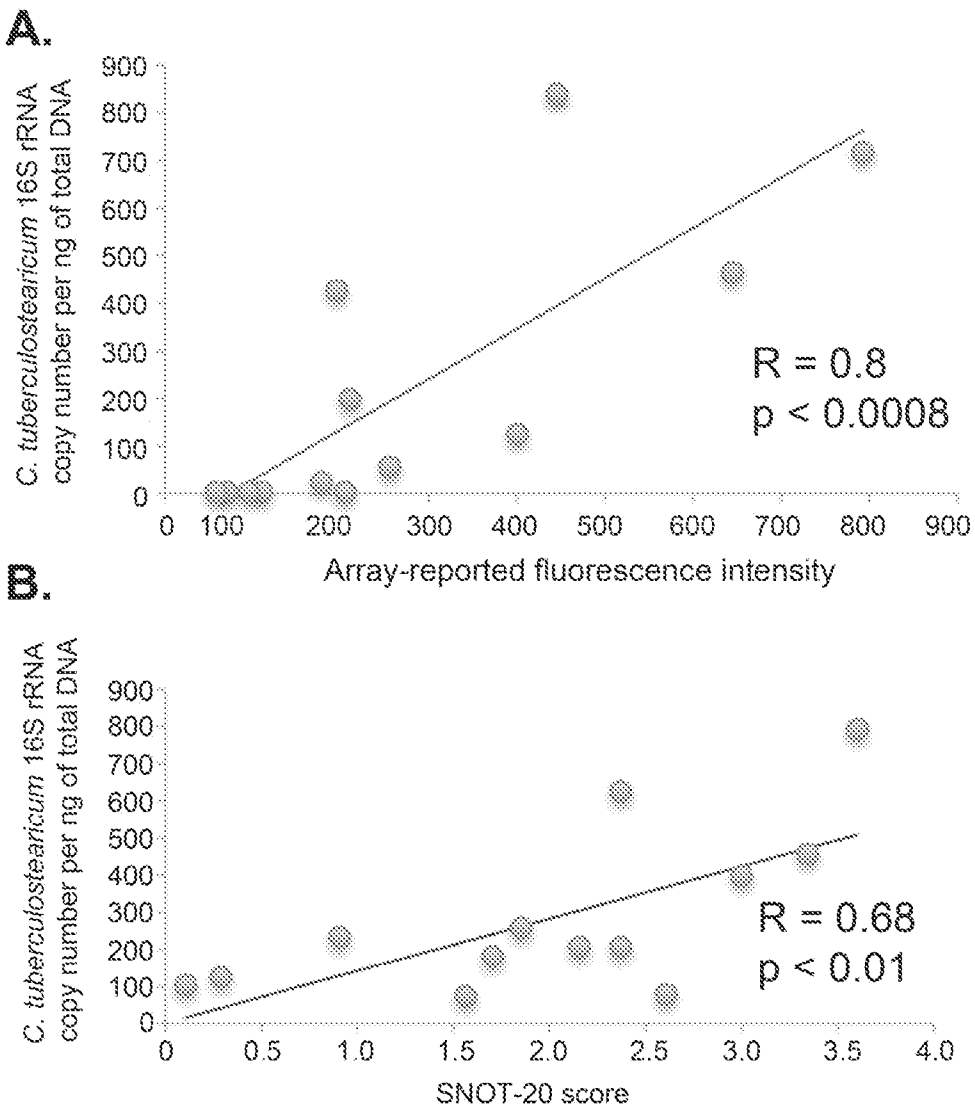

A.
C. tuberculostearicum 16S rRNA copy number per ng of total DNA
900
800
700
600
500
400
300
200
100
0
0 100 200 300 400 500 600 700 800 900
Array-reported fluorescence intensity
R = 0.8
p < 0.0008
B.
C. tuberculostearicum 16S rRNA copy number per ng of total DNA
900
800
700
600
500
400
300
200
100
0
0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0
SNOT-20 score
R = 0.68
p < 0.01

FIGURE 5

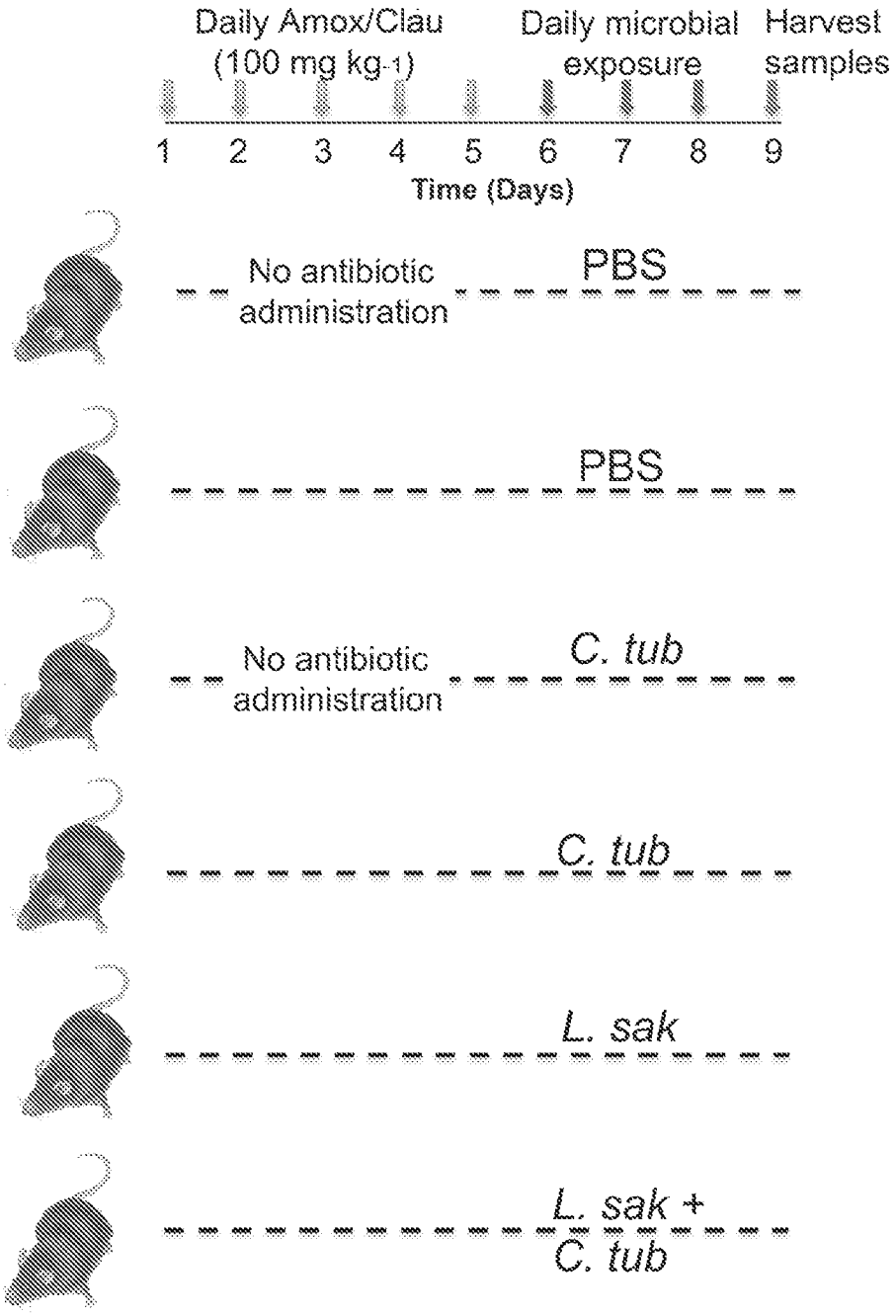
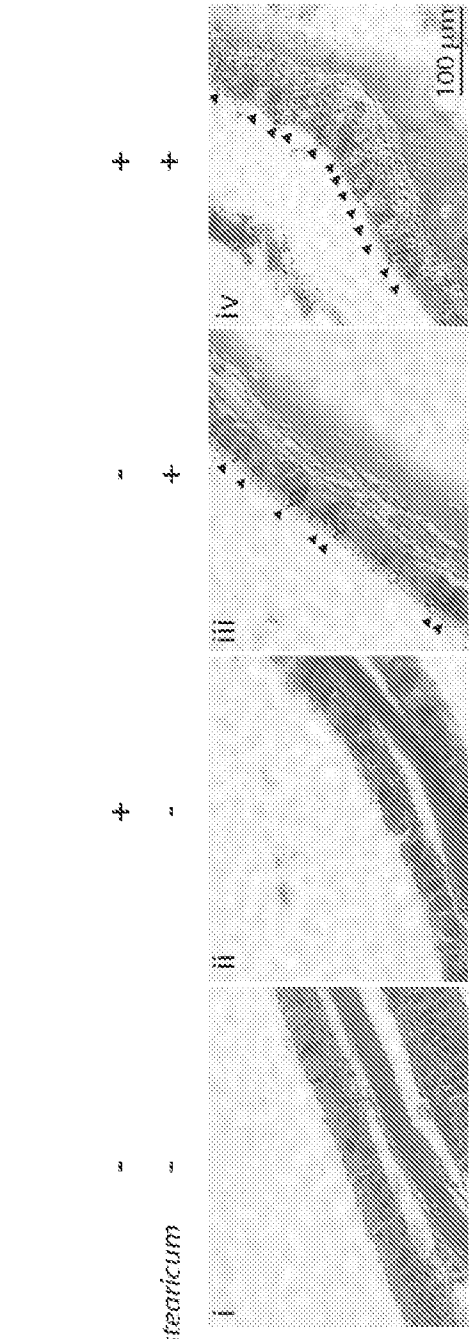

Daily Amox/Clau
(100 mg kg-1)
Daily microbial
exposure
Harvest
samples
1 2 3 4 5 6 7 8 9
Time (Days)
No antibiotic
administration
PBS
PBS
No antibiotic
administration
C. tub
C. tub
L. sak
L. sak +
C. tub

FIGURE 6


A.
Antibiotic - + - +
C. tuberculostearicum - - + +
i
ii
iii
iv
100 μm

B.
Goblet cell number per μm sinus epithelium
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
p < 0.003
p < 0.0001
p < 0.0007
AB-CT-
AB+CT-
AB-CT+
AB+CT+

FIGURE 7

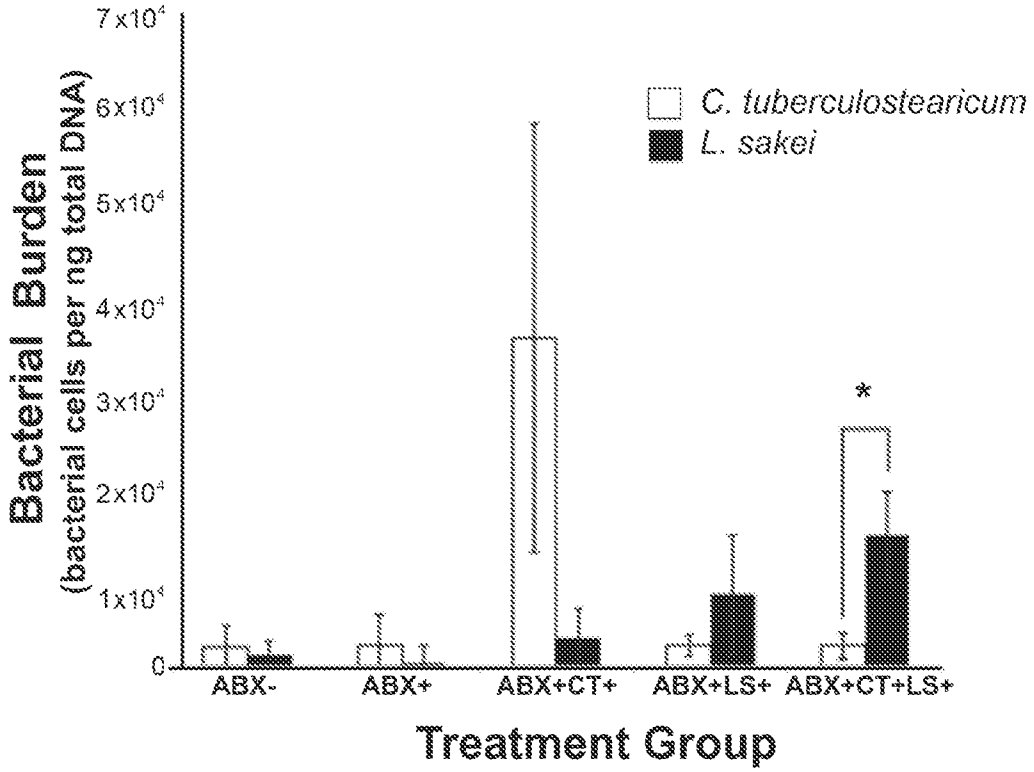
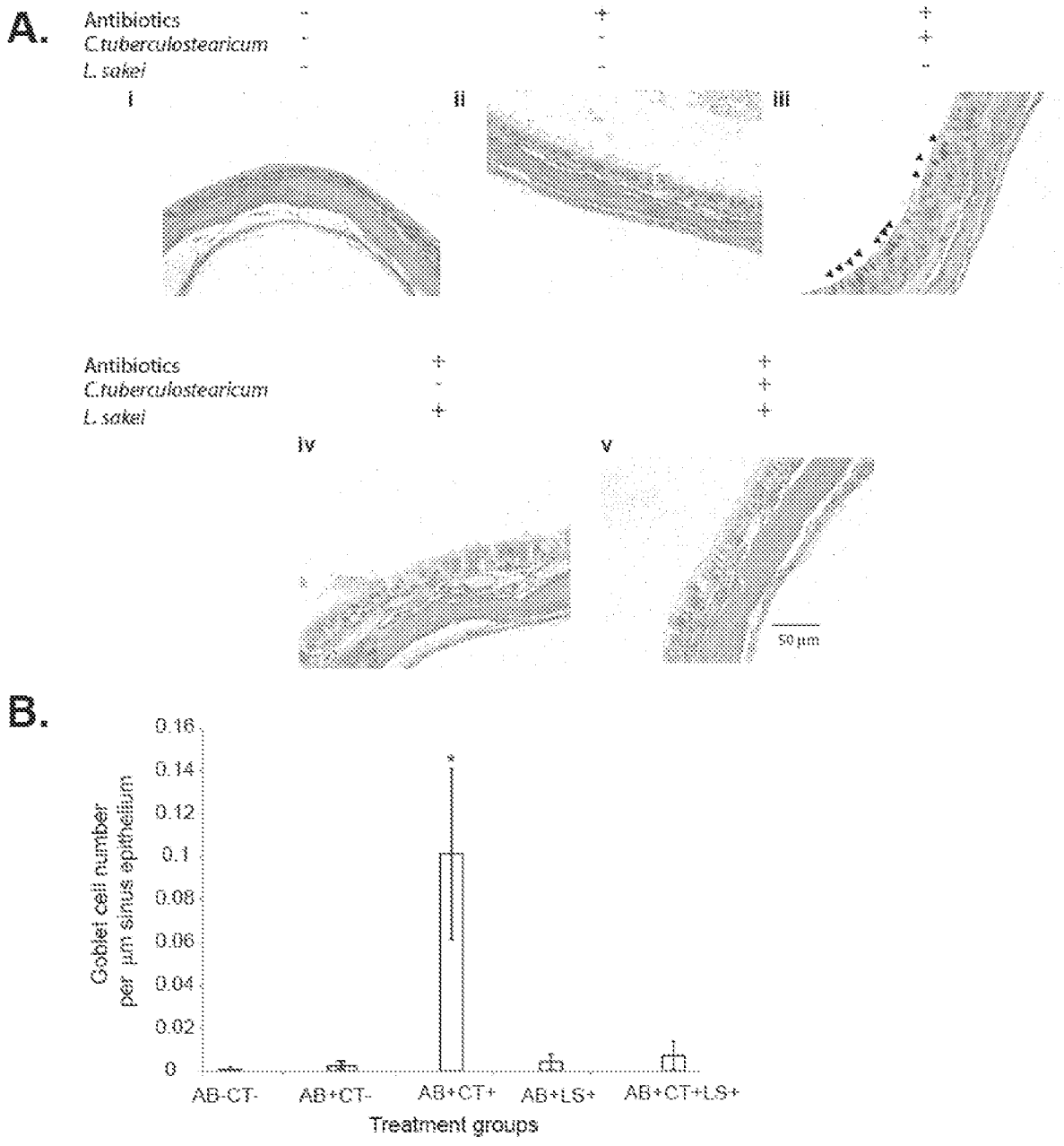

Bacterial Burden
(bacterial cells per ng total DNA)
7x10⁴
6x10⁴
5x10⁴
4x10⁴
3x10⁴
2x10⁴
1x10⁴
0
C. tuberculostearicum
L. sakei
*
ABX-
ABX+
ABX+CT+
ABX+LS+
ABX+CT+LS+
Treatment Group

FIGURE 8


A.
Antibiotics
C.tuberculostearicum
L. sakei
i
ii
iii
Antibiotics
C.tuberculostearicum
L. sakei
iv
v
50 µm
B.
Goblet cell number per µm sinus epithelium
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
AB-CT-
AB+CT-
AB+CT+
AB+LS+
AB+CT+LS+
Treatment groups

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/036297

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 35/74
USPC - 424/93.45
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 33/00, 35/66, 35/74, 39/02; A61P 11/02; G01N 33/569, 33/68 (2013.01)
USPC - 424/92, 93, 93.45; 435/252.9

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 9/0043, 31/09, 33/00, 35/74, 35/741; C12Q 1/04, 1/6888

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Patbase, Google Patents, Google, Pubmed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2012/019058 A1 (HAM-MARSTROM et al) 09 February 2012 (09.02.2012) entire document	9-11 --- 2-4, 12, 14, 15
X -- Y	JOUSIMIES-SOMER et al. 'Comparison of the Nasal Bacterial Floras in Two Groups of Healthy Subjects and in Patients with Acute Maxillary Sinusitis.' Journal of Clinical Microbiology. Vol. 27: No 12. Pages 2736-2743. December 1989. entire document	20, 22 --- 21
Y	WO 2000/78322 A2 (DE SIMONE) 28 December 2000 (28.12.2000) entire document	1-4
Y	FOKKENS et al. 'European position paper on rhinosinusitis and nasal polyps.' Rhinology. Official Journal of the European and International Societies. Vol. 50: Supplement 23: Pages 1-329. March 2012. entire document	1-4, 13-16
Y	US 2010/0189702 A1 (DRAKE et al) 29 July 2010 (29.06.2010) entire document	13-16
Y	BERNARD et al. 'Cellular Fatty Acid Composition as an Adjunct to the Identification of Asporogenous, Aerobic Gram-Positive Rods.' Journal of Microbiology. Pages 83-89. January 1991. entire document.	21
Y	US 2009/0214497 A1 (PARK et al) 27 August 2009 (27.08.2009) entire document	12, 16

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 July 2013	29 JUL 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/036297

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/036297

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-8, 17-19, 23-27
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.