

# Novel Microbiome-Based Therapeutics for Chronic Rhinosinusitis

E. K. Cope · S. V. Lynch

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**Abstract** The human microbiome, i.e. the collection of microbes that live on, in and interact with the human body, is extraordinarily diverse; microbiota have been detected in every tissue of the human body interrogated to date. Resident microbiota interact extensively with immune cells and epithelia at mucosal surfaces including the airways, and chronic inflammatory and allergic respiratory disorders are associated with dysbiosis of the airway microbiome. Chronic rhinosinusitis (CRS) is a heterogeneous disease with a large socioeconomic impact, and recent studies have shown that sinus inflammation is associated with decreased sinus bacterial diversity and the concomitant enrichment of specific sinus pathogens. Here, we discuss the potential role for probiotic supplementation for CRS in light of this increasing understanding of the airway microbiome and microbial interactions with the host. We focus on the ecological significance of microbiome-based probiotic supplementation and potential interactions with the gastrointestinal tract and consider microbial administration methods for treatment of CRS.

**Keywords** Microbiome · Airway · Respiratory tract · Chronic rhinosinusitis · CRS · Sinusitis · Probiotic · GI-respiratory axis · Lactobacillus

## Introduction

The human microbiome, the collection of microbial species and their associated genomes that live on and in the human

body, are remarkably diverse [1]. Though a relatively new field, technological advances have led to a rapid increase in our understanding of these communities. Each healthy individual houses multiple distinct microbiomes at different body sites; though inter-individual variation exists, the composition of the microbiome at any given site is broadly similar across healthy subjects. As would be predicted from traditional single species microbiological studies, these communities of microbes interact extensively with the host [2–4]. Our resident microbial pan-genome outnumbers that of humans by at least 100× in terms of functional gene content, providing a functional repertoire that contributes metabolic capacity, drives immune development, and affords mucosal protection [5–7]. Perturbations to these microbial ecosystems are increasingly associated with a myriad of inflammatory and autoimmune diseases of the gastrointestinal (GI) tract, airways, and skin [8–11]. Recent success in transplanting healthy gut microbiota to patients with refractory *Clostridium difficile* colitis [12] has fueled interest in the development of rationally designed microbiome supplementation strategies for application in other inflammatory diseases, including chronic rhinosinusitis (CRS). Here, we discuss the potential for probiotic for treatment of CRS in light of recent developments that have enhanced our understanding of the human microbiome.

## The Microbiome in Airway Disease

### Physical Environment Along the Respiratory Tract

The respiratory tract is in constant contact with inhaled pollutants, allergens, and microbes [13, 14]. This inhaled debris is cleared from the sinonasal cavity, nasopharynx, trachea, and lungs through interaction between ciliated epithelia and mucus producing goblet cells—a mechanism termed mucociliary clearance [14]. Non-keratinized stratified squamous epithelia line the oropharynx and laryngopharynx, while the nares are lined by keratinized, stratified squamous epithelia with

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E. K. Cope  
Department of Otolaryngology Head and Neck Surgery, University of California San Francisco, San Francisco, CA 94143, USA

S. V. Lynch (✉)  
Division of Gastroenterology, Department of Medicine, University of California San Francisco, San Francisco, CA 94143, USA  
e-mail: Susan.Lynch@ucsf.edu

sebaceous glands, sweat glands, and hair. These physiologically distinct niches offer discrete ecosystems for microbial colonization and are colonized by a compositionally distinct bacterial microbiome [15]. In addition to mucociliary clearance, airway epithelia express pattern recognition receptors (PRRs), such as toll-like and bitter taste receptors [16], that can recognize and respond to microbes [17], leading to the recruitment of immune response cell subsets such as neutrophils, eosinophils, and/or macrophages and epithelial secretion of antimicrobial peptides, chemokines, and cytokines [18–20].

#### *Chronic Airway Diseases Are Associated with Aberrant Microbiota*

As one of the primary points of contact with environmental microbes, it is unsurprising that the healthy respiratory tract, particularly the upper respiratory tract, is colonized by a diverse microbiome [21•]. In healthy subjects, the burden of microbes and diversity of the airway microbiome are greatest in the upper airway and diminish upon progression towards the lower airway. It remains unclear whether the microbial communities detected in the most distal airspaces of healthy subjects colonize the mucosal surface or represent transient inhaled species. In pulmonary diseases, such as asthma, cystic fibrosis (CF), and chronic obstructive pulmonary disease (COPD), the lower airways are overtly colonized by microbes [10, 22]. In the case of the genetic disease, CF, airway infections were historically associated with single pathogens; however, culture-independent studies have identified polymicrobial pathogenic microbiota in these patients. More recent studies have indicated that the lower airway microbiomes present in pediatric CF patients are much more diverse than those found in adult patients [10, 23–28]; over 60 distinct taxa have been identified in sputum from children with CF [28, 29]. As patients' age and pulmonary disease progresses, lower airway microbiome communities become less diverse and are dominated by pathogenic taxa, which are refractory to antimicrobial treatment [10, 25, 30, 31]. Environmental exposures may also play a role in lower airway microbiome composition in CF patients; CF sibling pairs share a larger proportion of their microbiota than non-sibling CF patients, suggesting that shared environmental exposures impact development of the lower airway microbiota in these patients [30].

Microbiome dysbiosis in the lower airways has been implicated as a driving or exacerbating factor in the pathophysiology of asthma. Asthma is a heterogeneous inflammatory disease characterized by reversible bronchial constriction and Th2 hyper-responsiveness and is a common co-morbidity of CRS patients with nasal polyps. The composition of the bronchial and oropharyngeal microbiota is altered in patients with sub-optimally controlled asthma and track with features of the disease such as the degree of bronchial hyper-

responsiveness, suggesting that these perturbed airway microbial communities contribute to asthma pathogenesis [22, 32, 33]. There is also a growing body of evidence that early-life microbial exposures, specifically those known to impact the microbiota, can lead to increased risk of asthma and allergic diseases [34–36]. Recent studies have demonstrated that anti-microbial ablation of the microbiome enhances susceptibility to experimental allergic asthma in mice [37•]. In a recent study, gut microbiome manipulation achieved through oral administration of *Lactobacillus johnsonii* induced airway protection against airway cockroach and ovalbumin allergen challenge in mice. Protection was associated with reduced Th2 cytokine expression, reduced activation of CD11c+/CD11b+ and CD11c+/CD8+ dendritic cells, and altered gut microbial composition [38•]. Taken together, common themes in airway diseases are emerging. Microbiota are present in the respiratory tract in disease states, and changes in microbiome composition are coupled with alterations in the respiratory tract environment, e.g., mucin production and immunologic dysregulation. What has also emerged is strong evidence for the existence of a gut-airway axis, indicating that microbiome manipulation-based therapies may need to consider both the respiratory and gastrointestinal microbiomes to afford a more fulminant protective effect in patients with chronic respiratory diseases.

#### *The Sinus Microbiome in Health and Disease*

Similar to other chronic inflammatory diseases, including inflammatory bowel disease and asthma, evidence is emerging for the role of the sinus microbiome in defining upper airway health. CRS is a large public health problem, costing up to \$8.6 billion each year in lost productivity and treatment [39, 40]. Currently, there are conflicting reports of the role of the microbiome in CRS; however, these differences are likely due to intrapersonal variation of the sinus microbiota shaped by environmental exposures and geography, as well as the variation in sampling techniques and approaches to assay and analyze the microbiome. Despite this, two trends in the literature are evident. First, all three studies that have examined the microbiota of healthy subjects demonstrate the presence of a diverse microbiome that includes bacterial groups classically considered as causative agents of respiratory disease, including *Pseudomonas*, *Staphylococcus*, and *Streptococcus* [41•, 42, 43]. Second, substantial sinonasal microbiome dysbiosis is associated with CRS [42, 44]. In one example, Abreu and colleagues demonstrated microbiome community collapse in the maxillary sinuses of CRS patients compared to healthy controls characterized by the outgrowth of *Corynebacterium tuberculostrictum* [45]. In another study, nasal lavage specimens from CRS patients revealed microbiome collapse coincident with *Staphylococcus* enrichment [46]. Specimens from the middle meatus of patients with CRS did not overtly

demonstrate this pattern of microbiome diversity loss [43], but they did report a significant enrichment of *Corynebacterium*, *Pseudomonas*, *Staphylococcus*, and *Curtobacteria*. Differences in microbiome structure may be due to microbial biogeography, i.e., sampling of sinus vs. middle meatus, the tool, or techniques used to obtain specimens, e.g., brush vs. swab, or due to primarily sampling the mucus-associated microbes rather than the mucosal-adherent communities. In addition, the impact of other factors that may influence the sinus microbiome, such as concomitant lower airway disease, smoking, and treatments (antibiotic and/or corticosteroid use), is of scientific interest but are currently poorly defined in this patient population.

Immune responses in individuals with CRS vary considerably across patients [47]. Although the presence of polyps is typically used to phenotypically stratify patients, polyps can arise via different pathophysiologic mechanisms and may primarily be eosinophil- or neutrophil-derived [48]. Moreover, a variety of innate and adaptive immune components contribute to CRS-associated inflammation, which varies across patients regardless of polyposis. Epithelial cell production of antimicrobial peptides [49]; local IgA and IgE biosynthesis in response to specific microbial antigens [50];  $T_H1$  (IFN- $\gamma$ ),  $T_H2$  (IL-4, IL-5, IL-13), or  $T_H17$ -skewed inflammatory processes [51]; loss of epithelial barrier integrity [52]; and the more recently described group 2 innate lymphoid cells [53] variably contribute to CRS pathogenesis. While the underlying processes contributing to a patient's immune response are not well understood, there is evidence for microbial stimulation. *Staphylococcus aureus* exotoxins are associated with a  $T_H2$  inflammatory response characterized by eosinophilia and enterotoxin-specific IgE [50], and the  $T_H2$  cytokines IL-4 and IL-13 have been associated with *S. aureus* outgrowth in other inflammatory diseases [54]. Another common sinus pathogen, *Pseudomonas aeruginosa*, can induce antimicrobial nitric oxide production by host recognition of bacterial quorum sensing molecules through stimulation of the bitter taste receptor T2R38 [55]. There is clearly heterogeneity across patients with CRS; thus, future therapeutic microbiome manipulation strategies must be targeted to the specific microbiome perturbation and immune dysfunction of the patient. Though this may seem an insurmountable task, it is likely that CRS patients stratify into a reasonably small number of compositionally distinct pathogenic microbiomes. Leveraging this capacity to stratify patients based on their sinus microbiome composition and associated immune dysfunction offers the opportunity to more precisely tailor both existing therapeutics and those in development aimed at manipulating the sinus microbiome to restore immune homeostasis.

## Existing Therapies and Challenges in Treating CRS

Since CRS is immunologically and microbiologically diverse, it is not surprising that current treatment strategies using corticosteroids alone or in combination with antibiotics are variably successful. Some patients recover completely without recurrence, although 10–25 % of patients require repeated treatment. Culture-directed antibiotic use has shown some success [56]; however, recurrence of disease following antibiotic administration targeted to the cultured pathogen is still common [57]. Patients who do not respond to medical management are candidates for functional endoscopic sinus surgery (FESS). The goal of FESS is to remove polypoid tissue and open ostia to facilitate sinus drainage. While some patients rebuild their native, healthy microbial communities and epithelium following FESS, many patients require revision sinus surgeries [58]. Importantly, these therapies only manage chronic airway diseases and, in many cases, do not address the underlying source of disease, e.g., dysregulated microbiota. Since it is clear that the microbiome plays a fundamental role in respiratory health, it is essential to begin to define the interaction between pathogens or pathobionts in the context of the healthy host microbiota.

## Microbiome Restoration in the Airways

### *Probiotics, Prebiotics, and the Microbiome*

Disturbances to the sinus microbial ecosystem following antibiotic administration, acute infection, or persistent stresses, such as exposure to tobacco smoke, can degrade community composition, resulting in loss of critical commensal species and their functions necessary to activate immune homeostasis (Fig. 1). Due to competitive colonization, the pathogenic community that develops is frequently resistant to colonization by commensal sinus microbes. However, by applying ecological principles and leveraging existing ecosystem rehabilitation strategies at higher trophic levels, it is possible to identify community members, resources, or functional attributes in the sinus microecosystem that may be exploited for therapeutic purposes. Macroecosystem disturbance of, for example, forests, rivers, and lakes, mirrors those of the disturbed sinuses in that they are associated with species diversity loss and invasive species overgrowth. Efforts to drive macroecosystem rehabilitation following disturbance have focused on strategies to remove or decrease the abundance of invasive species. Such an approach provides a framework for rational design of therapeutic strategies to rehabilitate the sinus microbiome following disturbance.

Though the respiratory microbiome has only very recently been considered a potential strategic target for therapeutic manipulation, the gastrointestinal microbiome has been the focus of manipulation efforts for over 100 years. In the early

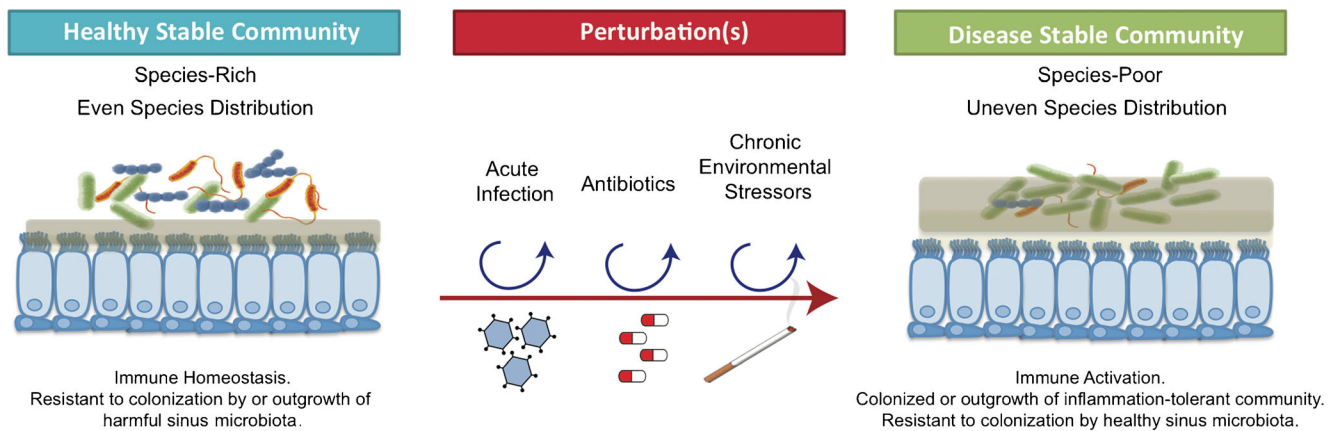


**Fig. 1** Secondary succession in the sinus microbiome. The healthy sinus microbiome is comprised of a diverse microbial community that interacts with the host epithelium to maintain immune homeostasis or outgrowth of

harmful sinus pathogens. Secondary succession following perturbations to this ecosystem can lead to a stable microbiome that has reduced diversity in favor of inflammation-tolerant pathobionts

1900s, Elie Metchnikoff described the use of microbial supplementation to treat enteric infection [59]. The modern era has seen unprecedented growth in the probiotic, prebiotic, and symbiotic supplement field. Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [60, 61]. *Lactobacillus* or *Bifidobacterium* spp. are primary species traditionally used in probiotic supplements and have exhibited variable success for a large number of disorders. This is likely due to the variety of strains with distinct functional capacities used in commercially available probiotic supplements, as well as the inherent variability in gut microbiome composition across consumers. Oral ingestion of *Bifidobacterium bifidum* MIMBb75 was successful in improving symptoms of irritable bowel syndrome in adults (IBS, [62]); however, this benefit was not observed when *Escherichia coli* Nissle 1917 was used [63]. The use of a wider breadth of species in probiotics appears to improve their efficacy, at least for specific indications. The probiotic VSL#3, a combination of seven bacterial strains comprising three genera, *Lactobacillus*, *Bifidobacteria*, and *Streptococcus*, is efficacious for improving ulcerative colitis disease symptoms [64] and inducing disease remission [65, 66]. The concept of more fulminant microbiome rehabilitation has gained traction following a report by Van Nood and colleagues. Fecal microbial transplantation for treatment recalcitrant *C. difficile* infection was approximately 90 % efficacious [12]. This approach involved infusion of a fecal slurry derived from a healthy donor to the distal intestine of the patient. This rate of efficacy has also been reported in pediatric *C. difficile* patients [67–69]. In attempts to refine this rehabilitation strategy, efforts involving encapsulated donor stool [70] or treating with a standardized mixture of common stool-associated bacteria [71] have been employed with variable success rates. These efforts described here provide an overview on probiotic manipulation of the gut microbiome. However, these strategies are eminently applicable to manipulating microbiome composition in other anatomical niches, not least the upper airways.

#### *Probiotic Efficacy for the Treatment of Airway Disease: GI-Respiratory Axis*

Murine studies have demonstrated that the GI microbiome plays a central role in shaping immune responses [5, 6]; hence, a large portion of immunological development is likely influenced by the developing gut microbiome in the first few years of life. Risk factors for the development of airway diseases such as childhood allergic asthma, include exposures such as antimicrobial administration, caesarian section, or formula feeding that are known to affect the composition of the neonatal gut microbiome [34, 36, 72]. As discussed above, adult patients with asthma and allergic rhinitis also exhibit altered airway microbiota [22, 33, 73]. Collectively, these observations suggest that microbiome disturbances at both local and distal sites may play a role in the development of allergic or inflammatory diseases of the airways and highlight distinct windows for probiotic intervention for the prevention or management of disease.

Support for this hypothesis comes from several probiotic supplementation studies targeting respiratory tract diseases [74–77]. Positive results reported from oral administration of probiotics [78, 79] provide evidence for a relationship between gut microbiome composition and airway health. For example, early-life exposure to microbiota in mice was protective against induced inflammatory bowel disease and asthma by preventing invariant natural killer T cell (iNKT) accumulation in the colon and lungs [80]. In a separate study, migration of  $CD3^+CD4^+IFN\gamma^+$  T cells from the intestine to the lung was induced by oral administration of *Lactobacillus rhamnosus* [76]. Thus, manipulation of the gastrointestinal microbiome in infancy or early childhood, as these assemblages develop, may represent a viable strategy probiotic intervention targeting respiratory diseases. Early-life exposure to livestock or furred pets (dogs and, to a lesser extent, cats) is associated with decreased risk for development of asthma or

atopy [81, 82]. This is believed to be due to infant exposure to environmental microbes associated with these animals. This hypothesis was supported by a mouse study of airway allergen challenge in which animals that were supplemented daily with house dust from a household with dogs present were protected against airway pathology [38•]. These animals also demonstrated altered gut microbiome composition and enrichment of a *Lactobacillus* species, which, when used to supplement mice, not only protect against airway allergen challenge but also protect the animals against airway infection with respiratory syncytial virus (39). The role for oral probiotic intervention later in life is more complex; there are limited clinical trials, generally in small cohorts (<100 individuals), which show a range from zero [83–85] to modest effects [83, 86–88] on symptoms of allergic rhinitis or asthma. It is well established that by 1–3 years of age, the gut microbiome reaches a more stable state and the immune system has reached major developmental milestones. In addition, most clinical trials use typical probiotic microorganisms (*Lactobacillus* spp. or *Bifidobacterium* spp.) via oral ingestion without regard to the relative composition of the diseased GI microbiome. The gut microbiome has been shown to change in composition based on anatomical site [3], ethnicity [89], and age [89–91], amongst other factors; however, these are not considered when designing probiotic supplements. Probiotic studies that consider these factors will likely have a more pronounced effect on responsiveness of airway disease.

#### Probiotic Use for Treatment of Rhinosinusitis: Considerations for Timing and Delivery Method

As discussed above, the most common route of probiotic delivery (oral) takes advantage of the GI-respiratory axis. In the only clinical trial of probiotic use in chronic rhinosinusitis, Mukerji and colleagues reported that oral administration of *L. rhamnosus* R0011 improved patient-reported symptoms of rhinosinusitis in the short term (<4 weeks), but not the long term (8 weeks). These results suggest a potential role for GI microbiome manipulation to affect the sinus immune response [92]; however, there has not been a follow-up study to further elucidate this role. Repeated dosing or inoculation with mixed species could improve these results.

Several variables should be considered when designing probiotics for potential treatment of sinus disease. The first consideration, the route of administration, will determine the mechanism of action of the probiotic. Oral probiotic supplements primarily affect the respiratory tract through translocation of microbial metabolites, cytokines, or immune cells to the airways via systemic circulation, while local delivery via sprays or nasal lavage will affect the sinonasal microbiota and local immune responses [38•, 93–95]. Topical delivery

requires an appropriate delivery technique and device and will depend on the surgical state of the sinus cavity. Fluid dynamics within the sinuses at the time of delivery (volume and pressure) will also impact the efficacy of the probiotic to reach the mucosal surface, where it would likely have the greatest influence by interacting with the host epithelium. This first variable, route of administration, will determine which probiotic species are used. A second consideration for probiotic development is whether to supplement with a single species or a mixed-species consortium. Single species or species mixtures can be selected based on how best to leverage the healthy microbiome. From an ecological perspective, the potential role of the probiotic(s) should be considered. For example, the specie(s) may function as keystone (a species that has a disproportionately large effect on the community), pioneer (species that are the first to colonize the niche after a disruption), or dominant species found in a healthy state (species with a relatively high abundance in a niche). Finally, the timing of probiotic administration is an important consideration. Should the probiotics be considered adjuvant to treatment or be used as pioneer species to drive microbiome reassembly after antibiotic administration or surgery?

#### Animal Models Provide Insights for the Use of Probiotics to Treat Rhinosinusitis

Animal models are powerful tools for exploring the relationship of the host-microbiome to health and disease. Because of the complexity of host-microbiome interactions, animals are often used to determine the mechanisms by which microbiome dysbiosis drives phenotypic immune responses as well as to reveal benefits of microbial-derived metabolites and protective microbial communities. In malnourished mice, nasal instillation of *Lactobacillus casei* can confer protection against pathogens by enhancing host innate immune response [96]. Malnourished mice had reduced TNF- $\alpha$  expression and impaired leukocyte recruitment resulting in susceptibility to *Streptococcus pneumoniae* infection. On supplementation with either heat killed or live *L. casei*; however, mice regained the ability to mount a local and systemic response against *S. pneumoniae* infection. Live *L. casei* had additional benefits of temporarily colonizing the respiratory mucosa to competitively exclude *S. pneumoniae* [96, 97]. Intranasal administration of *Lactobacillus plantarum* DK119 protected mice from lethal loads of influenza A virus through modulating host immunity of alveolar dendritic cells and macrophages [98]. Similarly, intranasal administration of *L. rhamnosus* GG protected mice from H1N1 influenza infection by activating lung natural killer cells [99]. More recently, Fujimura and colleagues showed that supplementation of mice with dog-associated house dust protects against airway allergen challenge by reducing airway Th2 gene expression. They also show that this protection can be achieved through feeding a single species,

*L. johnsonii*, which was enriched in the cecum of mice fed house dust. Supplementation with *L. johnsonii* led to reduction in the number of CD11c+/CD11b+ and CD11c+/CD8+ dendritic cells, key antigen presenting cells in the gastrointestinal tract [38•]. In a sinusitis model, Abreu and colleagues demonstrated that intranasal administration of *Lactobacillus sakei*, identified using 16S rRNA phylogenetic microarray analysis of healthy human sinuses, protects against *C. tuberculo*stearium-induced sinusitis [45]. A similar murine study showed that *Staphylococcus epidermidis* can protect against *S. aureus*-induced sinusitis [100]. Together, these studies show promise for microbiome-based therapeutics in sinusitis. However, we must think critically about the species or community used for sinus protection, administration methods, as well as the timing for microbial intervention.

### Mechanisms of Probiotic Benefit to the Host

There has been a steady interest in understanding the role for probiotics in human health; however, we have only begun to grasp the complex interactions with our own microbiomes and immune system. As discussed above, transplantation of fecal microbial contents successfully treated recalcitrant *C. difficile* infection [12]. Normally present in the gut at low abundance, *C. difficile* takes advantage of a diversity-depleted microbiome to proliferate despite subsequent antimicrobial treatments. In this case, *C. difficile* can be considered an invasive species. By replenishing the microbiome with a diversity of organisms found in a healthy stool sample, it is possible to restore the system to a balanced ecological state. While this approach demonstrates the potential for microbial supplementation, the mechanisms by which the transplanted or supplemented microbes change the host ecosystem are not well understood.

### Probiotic-Microbiome Interactions

Probiotic administration can influence the host-microbiome composition and function directly through production of antimicrobials, changing the pH, or through competitive colonization within a niche. Bacteriocins are antimicrobial peptides produced by bacteria with a wide range of activity, either narrow spectrum (active against similar species) or broad spectrum (active across genera) [101, 102]. Lactic acid bacteria are well-established producers of bacteriocins. The protective species identified by Abreu and colleagues, *L. sakei*, is known to produce several bacteriocins with a wide range of characteristics and putative modes of action [45, 103], although the best characterized bacteriocin from this species is sakacin. Sakacin has antimicrobial activity against Gram-positive taxa, including *Listeria* spp. and *Enterococcus* spp., but not Gram-negative bacteria [104].

Other *Lactobacillus* species that are potential probiotics for the airways act through the production of alternative antimicrobial compounds. *Lactobacillus reuteri* produces the protein reuterin [105], which acts as an antimicrobial compound by inducing oxidative stress in competing bacteria [106]. Reuterin production is increased in the presence of *E. coli*, suggesting that the effects of this protein are aimed at eliminating competing microbes, giving *L. reuteri* an advantage in adherence and colonization of host mucosa [106]. *Lactobacillus* spp. also commonly produce acetic acid and lactic acid, thereby lowering the pH of their niche and inhibiting the growth of acid-intolerant taxa [107–109]. Finally, probiotic species can compete for growth substrates or receptor binding sites. *L. johnsonii* competes with several known pathogens for adhesion receptors, which are either glycoproteins or glycolipids [110]. One such receptor is ganglioside GM1 (asialo-GM1), a glycolipid that is abundant in pulmonary tissue [111]. Several pathogens associated with airway disease, *P. aeruginosa*, *Haemophilus influenzae*, and *S. aureus*, are known to bind specifically to asialo-GM1 receptor, suggesting that probiotic taxa with the ability to bind this host ligand may competitively bind available asialo-GM1, thus reducing the burden of pathogenic colonizers in the airways.

### Probiotic-Host Interactions

Microbial products and metabolites have a profound influence on the host. Polysaccharide A (PSA), a capsular molecule produced by the gut commensal *Bacteroides fragilis*, influences the development of Foxp3+ Treg cells in the GI tract, which produce the anti-inflammatory cytokine interleukin (IL)-10 [112]. Gastrointestinal exposure to PSA has also been shown to influence IL-10 expression in peripheral tissues, including the lungs, and has a significant protective effect against experimental asthma in mice [113]. Oral administration of *B. fragilis* can have far-reaching effects on the host; this organism can restore GI epithelial barrier function, preventing leakage of harmful molecules from the GI tract to the serum, which can affect host behavior [7].

Microbial metabolites are also implicated in host-microbe cross talk. Specifically, short-chain fatty acids (SCFAs), which are breakdown products of dietary fiber, have a large influence on gut immune homeostasis. Microbial-derived butyrate, a common SCFA, has anti-inflammatory effects on the GI epithelium through regulation of NF-κB activation [114] as well as by influencing the differentiation and expansion of colonic Treg cells [115]. A specific clade of commensal *Clostridia* present in the healthy gut microbiome can induce CD4+ FOXP3+ Treg differentiation driven by microbial production of SCFAs, which is protective against models of colitis and allergic diarrhea [116•].

## Conclusions

Probiotic intervention for respiratory diseases is an area of active investigation, particularly in light of recent microbiome findings. While the field is still relatively nascent, the potential for probiotic manipulation of the sinus microbiome to treat or prevent CRS is great. However, our current understanding of the healthy sinus microbiome and, thus, how best to manipulate it in a disease state are not well defined. Whether to use mixed versus single species and strain inocula, specific species used, mode of delivery, inoculum concentration, and determining the frequency of supplementation are some of the factors that need to be addressed in optimizing probiotic effects [117, 118]. Most of the studies discussed in this article have focused on the gut microbiome and effects at distal sites because these interactions have formed the focus of the majority of studies to date. However, the murine studies discussed here suggest that local administration of probiotics to the sinuses can affect the dynamics of the sinus microbiome. Studying whether single keystone taxa or a consortium of carefully chosen microbiota has greater efficacy in treating rhinosinusitis would be one of the first steps toward developing a sinus-specific probiotic. Determining the mechanism of action, that is, exactly what the probiotic-microbiome and probiotic-host interactions are will allow us to further refine our supplementation strategies toward broadly useful treatment regimen. Since there is a clear role of microbiome dysbiosis in CRS, either in driving or exacerbating disease, we propose that future studies should focus on the rational design and administration of probiotics as an option for treating CRS.

## Compliance with Ethics Guidelines

**Conflict of Interest** S.V. Lynch reports grants from Janssen Pharmaceuticals. In addition, Dr. Lynch has a patent 61/624 pending. E. K Cope declares no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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- Of importance
- Of major importance

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