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Rational identification of diet-derived postbiotics for improving intestinal microbiota function

Cory Klemashevich[1], Charmian Wu[2], Daniel Howsmon[1], Robert C Alaniz[3], Kyongbum Lee[2] and Arul Jayaraman[1,3]

The intestinal microbiota plays an important role in a wide range of functions and whole body homeostasis. Recent advances have linked microbiota dysbiosis to conditions ranging from Crohn's disease to cancer. The restoration or strengthening of the intestinal microbiota through diet-based approaches such as probiotics and prebiotics has been proposed for combating the onset or progression of these diseases. In this review, we highlight the importance of postbiotics for the manipulation of the intestinal microbiota, with special emphasis on systems biology computational tools and targeted metabolomics for the rational discovery and identification of these bioactive molecules. The identification of novel postbiotics and the pathways responsible for their production should lead to improved mechanistic understanding of the role that specific probiotics, prebiotics, and postbiotics have in restoring intestinal microbiota composition and function.

Addresses
[1] Department of Chemical Engineering, Texas A&M University, College Station, TX, United States
[2] Department of Chemical and Biological Engineering, Tufts University, Medford, MA, United States
[3] Department of Microbial and Molecular Pathogenesis, Texas A&M University Health Science Center, College Station, TX, United States

Corresponding authors: Jayaraman, Arul (arulj@tamu.edu, arulj@mail.che.tamu.edu)

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Introduction

The human gastrointestinal (GI) tract is colonized by $\sim 10^{14}$ bacteria belonging to ~1000 species that are collectively termed the intestinal microbiota [1]. This bacterial community is important for digesting dietary nutrients and extracting energy by fermenting carbohydrates indigestible by human enzymes to short-chain fatty acids (SCFAs) [2]. Recent studies show that the intestinal microbiota also impacts a wide range of functions in the GI tract including development of the immune system [3], defense against pathogens [4], and inflammation [4]. Beyond the GI tract, gut-brain [5], gut-lung [6•], and gut-liver [7] links have also been identified, highlighting the importance of the microbiota. Consistent with the view that the microbiota is critical for whole body homeostasis, microbiota-derived metabolites have been detected in circulation [8], and alterations in the intestinal microbiota composition and function (i.e. dysbiosis) have been correlated to several diseases including obesity [9,10••], diabetes [11], cancer [12••], and asthma [6•]. Therefore, an emerging approach for combating the onset or progression of these diseases is the restoration or 'strengthening' of the intestinal microbiota [13–15].

Introducing probiotics (i.e. 'beneficial' bacterial species such as *Bifidobacterium bifidum*) or adding prebiotics (e.g. fructooligosaccharides [13]) that promote growth and activity of certain bacterial species are the conventional methods for manipulating the intestinal microbial community. Advances in high-throughput sequencing and metabolomics have led to the emergence of postbiotics that can be used to directly and specifically manipulate microbiota function. The primary aim of this review is to discuss these emerging trends for the manipulation of the intestinal microbiota through the diet. Since identifying the bioactive products of dietary molecules generated by the microbiota is non-trivial due to the biochemical diversity of the microbiota, we highlight the role that systems biology computational approaches and targeted metabolomics can play in the discovery and characterization of novel postbiotics.

Manipulating microbiota composition and function: state-of-the-art

Acute dysbiosis of the microbiota is typically associated with non-life threatening symptoms and does not necessitate treatment with high risk drugs [16], whereas chronic dysbiosis has been linked to more serious diseases such as type 2 diabetes [17•] and cancer [18]. Both prebiotic and postbiotic foods are becoming viable treatments for microbiota dysbiosis, with a particular focus on alleviating acute dysbiosis. Probiotic administration upon dysbiosis has been shown to partially restore the bacterial metabolic profile in addition to ameliorating antibiotic associated diarrhea in infants [19]. Introduction of *B. bifidum* in mice alleviated symptoms of irritable bowel syndrome (IBS) [20]. Similarly, co-administration of *Lactobacillus casei* with chronic low-dose aspirin treatment was effective

in reducing small bowel injury to IBS afflicted patients [21]. Probiotics have also shown promise as a possible treatment for mild alterations in behavior due to stress. Ingestion of *Lactobacillus rhamnosus* regulated the emotional behavior and γ-aminobutyric acid receptor expression in mice, suggesting that this microbe is a potential probiotic [22•]. Despite these advances, the FDA has yet to approve any health claims made by probiotics due to current poorly defined regulations [23].

Prebiotic molecules that promote the colonization and growth of beneficial bacteria in the GI tract have been regarded as viable candidates for dietary supplementation. A recent study in mice on a high-fat diet showed that polyphenol-rich extracts of pomegranate increased the cecum load of *Bifidobacterium* spp. and significantly reduced inflammation markers in the colon and visceral adipose tissue [24]. A study on the effects of tea extracts containing polyphenols reported that the prebiotic inhibited the growth of pathogenic *Clostridium* strains while simultaneously improving growth of non-pathogenic *Clostridium* strains [25]. In mice, fructooligosaccharide supplementation elicited anti-inflammatory and anti-allergic responses, and also improved stress resistance [26–28].

A major obstacle in the development of effective probiotic strains and prebiotics is the incomplete characterization of the intestinal microbial community under both homeostasis and disease states. While phyla level changes in the composition of the community have been documented, specific alterations at the species level remain unclear. Although a variety of food additives or supplements have been used to alter the microbiota composition [29,30•], the limited knowledge on the microbiota has hindered the development of rationally designed approaches (i.e. specifically targeting a particular group of bacteria and/or its function).

Further, incomplete information on intestinal microbial communities limits our understanding of how community level interactions will affect prebiotic treatments. It is well established that the spatial distribution of bacteria in the GI tract is heterogeneous [31,32], leading to different prebiotic activities at different locations. Since molecules produced by one bacterial species can be modified by other species in the local microenvironment, community-level biotransformation reactions could be necessary to increase the availability of a desired molecule in an active form [33]. Without cooperative interactions, a probiotic strain may not yield a beneficial effect, or the bioactive form of a prebiotic may never reach the intended target site, as has been demonstrated with the polyphenol quercetin [33,34]. While there have been promising advances in characterizing intestinal microbial communities, further progress will be necessary to gain a mechanistic understanding for many probiotic treatments, and thereby develop effective probiotic strains and prebiotics.

Putting the microbiota to work: diet-derived postbiotics

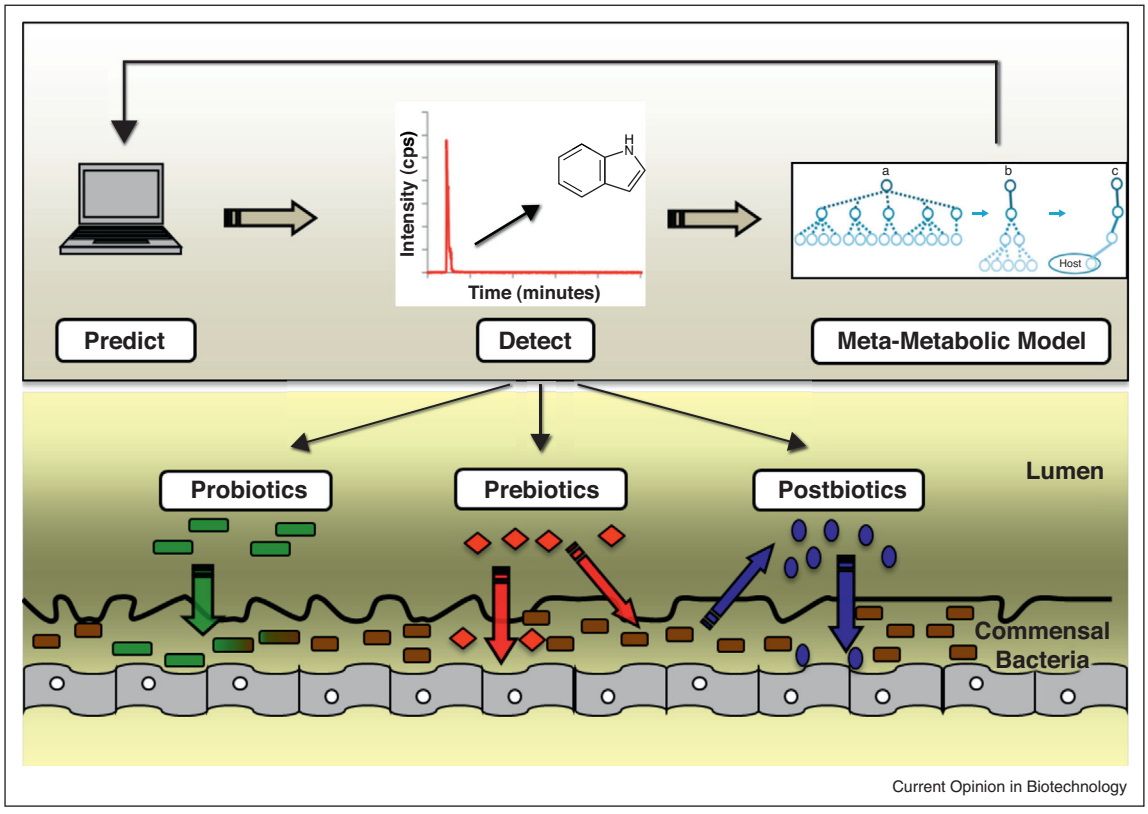
An emerging approach to strengthening the microbiota is to first identify the molecules that are depleted in a particular disease, and then supplement the diet with either the depleted molecule or a precursor molecule that can be converted to the bioactive molecule by the microbial community. This approach is especially attractive as these postbiotics are an important class of functional molecules used by the microbiota to modulate human health. Recent studies have investigated the fate of diet-derived postbiotics in the GI tract, and their impact on the metabolic profile of the microbiota in relation to disease [33,35,36].

Amino acid derivatives transformed by the gut microbiota make up one class of compounds that are potential postbiotics. For example, indole, which can be derived from tryptophan, is a possible link to microbiota dysbiosis as indole concentrations in fecal samples are reduced in patients suffering, but not recovering, from ulcerative colitis [37]. Bansal *et al.* showed that indole decreases indicators of inflammation, pro-inflammatory transcription factors, and pathogen colonization in intestinal epithelial cells while increasing tight junction resistance and mucin production, thus demonstrating indole as a postbiotic molecule [38]. SCFAs are another class of bioactive and beneficial molecules produced by the microbiota. A study comparing colonic microbes and their metabolites in human subjects of African origin with high and low risk for colon cancer found significant correlations between decreased production of SCFAs, increased levels of bile acid metabolites of bacterial origin and increased risk of colon cancer [39]. Changes in the abundance of butyrate, acetate, and propionate have also been correlated with health deterioration of elderly patients, further underscoring the importance of bacterial SCFA production in GI tract physiology [40••].

Toward rational identification of postbiotics

Metabolomics is a powerful approach for detecting and quantifying small molecules in complex biological systems, and thus well suited for the identification of postbiotics. A recent study by Kok *et al.* [41] used chromatographic separation coupled with tandem mass spectrometry (MS/MS) to characterize the impact of antibiotic treatment on the metabolite profile of rat urine samples. In a related study, Antunes *et al.* [42] detected more than 2000 metabolite features in murine fecal samples, and found that a single high dose of streptomycin caused significant changes in ~90% of these features. To date, most studies have utilized an untargeted approach to obtain a comprehensive profile of the altered metabolites. While this approach has discovery potential, it also has several drawbacks. Simultaneous quantification of a large number of metabolites using MS remains challenging due to the large dynamic range of metabolites

Figure 1



Rational identification of postbiotics. A comprehensive metabolic meta-model reflecting the biochemical diversity of the microbiota can be used in conjunction with targeted metabolomics to predict the metabolic derivatives of a dietary compound, identify prebiotics, or identify biotransformation pathways, given the detection of a postbiotic.

(up to 9 orders of magnitude [43]) and sensitivity limits. Without a predetermined set of targets, an extraction protocol and MS operating parameters cannot be tailored for particular classes of metabolites to improve sensitivity. Unambiguous identification of bacterial metabolites could also present challenges if the sample contains many ion fragments that have the same mass signatures.

The emergence of targeted MS [44] addresses some of these issues, as a focused set of target metabolites can be optimized for detection and quantitation while still broadly sampling the microbiota's biochemical diversity. In addition, computational tools have the potential for aiding targeted MS approaches and the identification of postbiotics. For example, metabolic models of the gut microbiome could be used to predict the possible metabolites of dietary compounds (e.g. polyphenols), and to identify which of these products are likely derived from the microbiota. In recent years, genome-scale metabolic models (GSMMs) have been published that describe the metabolic capabilities of specific human tissues under various physiological and disease states [45,46]. The human models have also been used as templates to reconstruct GSMMs for important model organisms, notably the mouse [46,47]. At present, a major bottleneck in constructing GSMMs is that the process is labor intensive, requiring substantial manual effort to curate the information found in genome annotation databases (e.g. KEGG), assuming that the species of interest have already been sequenced and annotated in the first place [48–50].

A notable effort in modeling the metabolism of intestinal microbiota is the work by Greenblum *et al.*, who assembled and analyzed a community level metabolic network model to find that there are topological differences (i.e. in the connectivity of metabolic reactions as defined by their stoichiometry) between normal, obese, and IBS afflicted patients [10••]. More recently, Heinken applied flux balance analysis (FBA) to study the metabolic interactions between a representative gut microbe, *B. thetaiotamicron*, and its murine host using a GSMM representing the two species [35,51].

In addition to constraint-based analyses such as FBA, another promising approach for studying community

level biotransformation using metabolic network models is computational pathway analysis. One example is PathMiner, which builds biotransformation pathways of a user-defined starting metabolite to minimize the overall energetic cost. Another algorithm, PathPred, exploits patterns of structural similarities between the reactants and products of biochemical reactions to predict potential degradation pathways. This algorithm is particularly useful for synthetic chemicals that are not natural substrates of known enzymes [52]. We developed ProbPath, which combines graph analysis and FBA to identify and rank possible synthesis routes for a user-defined target metabolite [53•]. In the context of predicting postbiotic metabolism, a useful feature of ProbPath is that it can selectively identify reactions that are non-native to the host organism, thus identifying metabolites that can be produced by one organism (e.g. a gut microbe) but not by another (e.g. human host). Presently, the most significant limitation of ProbPath is the dearth of appropriately constructed reaction databases for intestinal microbes. A promising meta-model was described by Ibrahim and Anishetty, who constructed a small-scale metabolic network (comprising 87 reactions) of human intestinal microbiota (based on species belonging to the three most abundant phyla) to study carbohydrate metabolism in the gut [54]. It should be emphasized that predictive methods and models are only as good as the accuracy and comprehensiveness of the available genome annotation data, and the lack of knowledge regarding the species comprising the microbiota has thus far limited *in silico* prediction.

Perspectives and conclusions

Manipulation of the microbiota through probiotics and prebiotics has shown great potential for treating a broad range of human diseases. Potentially more effective approaches, such as mixtures of prebiotics and probiotics, or 'synbiotics' [13], are emerging as our understanding of the role the microbiota plays in health and disease increases. A second emerging trend is the use of systems biology approaches to the rational development of prebiotics and postbiotics. While the identity and abundance of gut bacteria remain to be fully elucidated at the level of individual species, recent advances in metagenomics have begun to establish the phylogenies of microbes in human intestine [55•]. It is expected that the growing volume of genomic information will further accelerate the discovery of new probiotic strains as well as the development of food-derived prebiotics and postbiotics for treating human disease. Additionally, expanding the catalog of gut microbes with annotated genomes should facilitate systematic efforts to determine the likely reaction pathways used by bacteria to convert prebiotics into desired postbiotic metabolites. Prospectively, a comprehensive metabolic meta-model reflecting the biochemical diversity of the microbiota in conjunction with targeted metabolomics could be used to predict the metabolic derivatives of a dietary compound, identify prebiotics, or identify biotransformation pathways given a bioactive postbiotic (Figure 1). In turn, identification of bioactive microbiota metabolites and the pathways responsible for their production should lead to improved mechanistic understanding of the role that specific probiotics, prebiotics, and postbiotics have in restoring microbiota composition and function.

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