Title: Microbial activities are dependent on background conditions

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[Commentary for "How Causal are Microbiomes? A Comparison with the Helicobacter pylori Explanation of Ulcers" by Kate E. Lynch, Emily C. Parke, Maureen A. O'Malley]

Koch's postulates have been a useful tool in establishing a single – microbe causal explanation. This account connects the existence of an identified microbe to disease by repeatedly showing its isolation and successful infection. However, with the growing understanding of the microbiome's role in many physiological conditions, this simple notion of causal relations cannot capture the complexity of microbe-related diseases. Taking the case of H. pylori and ulcer, Lynch et al., demonstrate how framing Koch's postulate by an interventionist account clarifies the latter's explanatory strength in proportionality with the weaknesses in specificity and stability due to the factor of background conditions. They suggest this approach as an efficient way to bypass the enigma of background conditions and microbial interactions in the microbiome's causal relations. However, it is the background conditions and microbial interactions in the stomach that determine whether the presence of H. pylori results in an ulcer. I agree with their analysis of the problems and challenges with the microbiome causal explanation but argue that their suggested framework is insufficient without a proper examination of the background conditions and microbial interactions.

The microbiome functional properties are the result of their activity, interactions, and background conditions in addition to their taxonomic composition. Lynch et al. acknowledge this complexity but maintain the hope that a careful reductionist explanation is possible by looking at a core microbiome in connection to a properly define trait. I argue that adding the interventionist approach to Koch's postulates does not satisfy the microbiome causal explanation without considering the role of background conditions. The reductionist approach fits well within the model of causal explanation that centers on one causal entity. However, such a model is problematic because it does not address the factors of microbial activity and background conditions. I argue that for a better causal explanation with explanatory strength in specificity, it is essential to include the background conditions and microbial interactions. Furthermore, I argue that this inclusion changes the framework of the causal relations from looking for the *causal entity* to looking at a *causal process*.

The presupposition of a 'focal causal entity' stems from the Germ Theory framework, which attributes microbial properties of pathogenicity or non-pathogenicity independently of their background conditions. However, it has been shown that these microbial properties are also the result of processes of interactions and environmental conditions. I will show that focusing on the presence or absence of microbial composition as the causal entity delivers a causal explanation that is too broad. Therefore, following the background conditions and the microbial interactions is more than a methodological choice, it is the acceptance of the view of causal processes within the complex of their interdependence. This view needs further conceptualization of the microbial causal relations. Still, it is a preferable model for the causal explanations because it allows specificity.

Lynch et al. conceptualize four different ways by which scientists characterize the microbiome as 'compositional,' 'functional,' 'outcome-oriented,' and 'causal core' microbiomes (11). They distinguish between the 'compositional' and the 'functional' characterization by the fact that the taxonomic composition varies between individuals but, at the same time, have similarities in function. They explain that the functional characterization supposed to follow "the metabolic pathways and products of the microbiota," but in practice, "functions are typically inferred from genes in the microbiome sequence" (11). Furthermore, they explain that the taxonomic and functional composition goes together as the former identification "allows functional inferences about the metabolic and other biochemical pathways these taxa might [my underline] possess." (12). I argue that these functional inferences are not specific and hold the presupposition that the taxonomic composition has functional properties regardless of their activity and background conditions.

Firstly, inferring the microbial activity from "compositional characterization of microbes that focus on genes to identify taxa" (Lynch et al., 11-12) is too broad, leaving out the instances of gene expression in response to molecular interactions or metabolites composition in the environment. Such inferences are about the potential and possibilities of metabolism and other biochemical pathways, which makes them general inferences on the microbial activity that is not specific. Specificity is essential because of the dynamic nature of the microbiome's activity and interactions that also involve the background conditions. The microbial communities act and interact through their metabolic pathways, thus change their environment, which in turn affects their activity. Also, the microbial fermentation produces metabolites and other molecular signals, which regulates the host metabolism and immune response (Louis and Flint 2017; Rowland et al. 2018). Thus, the microbiome's activity should be examined carefully and not inferred solely by its taxa because of its dynamic, redundant, and mutuality with the environment (Ferrer et al. 2017; Rowland et al. 2018).

Furthermore, the taxonomic composition alone lacks information on the alterations in molecular agents such as gene expression, proteins, and metabolites (Ferrer et al. 2017). Thus, following the metabolic pathways can better explain the similarities in function across different taxa. The metabolic pathways have fluctuation depending on the abundance of nutrition and other environmental factors (Kim 2018). The metabolites are the products of gene and protein expression, which fluctuate due to environmental conditions and microbial interactions (Rowland et al. 2018). Such alterations affect the microbial activities and metabolites released to the environment. Therefore, to discuss the microbiome function without addressing the background environment lacks the explanation of the actual instances of the activity. Because of the bacterial interrelations within the gut is through fermentation, cross-feeding, and molecular signaling, the change in the production of metabolites is only partly the result of alteration in composition. It is also the result of changes in the fermentation pathways and consumption of the metabolites. Thus, the environmental condition, and bacterial interactions, together with their taxa, need to be considered in the causal explanation (Kim 2018).

For example, bacterial fermentation of carbohydrates produces short-chain fatty acids (SCFAs) and gases. SCFA are essential for the metabolism and gastrointestinal health of the host, and so it is important to associate which bacteria produce them. This identification can be made by following the key genes for specific metabolic activity of the bacteria. However, some bacteria can change their fermentation pathway and produce different SCFA under different substrate-dependent growth conditions (Rowland et al. 2018). Changes in the fermentation pathway, in turn, due to cross-feeding, can affect the abundance of other bacterial species, which in turn changes further the abundance of the metabolites in the environment. The

composition of the metabolites is essential for the understanding of physiological conditions because of their role in the regulation of various factors in the host, such as immune response, bacterial composition, and host metabolism (Rowland et al. 2018; Kim 2018). Thus, the background condition, in many cases, determines the bacterial properties and function and is also shaped by their interactions (i.e., the interactions modifies the background conditions of molecular and metabolites compound).

Secondly, Koch's postulates of causal explanations are framed by the Germ Theory and relay on the categorization of a single microbe as commensal, beneficial, or pathogenic regardless of its context (i.e., background condition and mutual interactions). Similarly, in the microbiome causal explanation, the focus is on the pathogenic/non-pathogenic properties of their taxonomic composition. This presupposition is problematic both in the case of *H. pylori*, as well as the more complex cases involving the microbiome. For example, polysaccharide A (PSA) released from *Bacteroides fragilis* affects the immune cells and their response to *Helicobacter hepaticus* in an animal model of experimental colitis (Round and Mazmanian 2009). The bacteria *H. hepaticus* is no longer pathogenic with the presence of *B. fragilis* because of the latter's release of PSA molecules to the gut environment affecting the immune response. Without the induction of PSA and its levels in the gut environment, this association in connection with the taxa gives different results.

The pathogenic/non-pathogenic categorization is problematic, especially if applying it to an entire microbiome (as Lynch et al. demonstrate in their analysis). The microbiome is an ecological community with microbial interactions that are heterogeneous with different functions and mutual causal directions (Mushegian and Ebert 2016; Skillings 2016). The fact that the host consists of various and interchangeable microbial communities with different patterns of interactions, transmission, and growth also challenges the idea of one criterion that can explain all instances of the immune response (Chiu and Eberl 2016). The bacterial communities in the host are heterogeneous, with various relations from symbiotic to pathogenic depending on their background condition and interactions (Ferrer et al. 2017). That is why the causal process of mutual interactions that considers the background condition is a better and more specific framework for the examination of causal relations. This framework includes the data on taxonomic composition but demands the additional inquiry which follows the microbial interactions.

Lastly, the microbiome study only flashes out the challenges to the causal explanation of diseases that were already present in the classical cases of single- microbe causal relations. The bacterial properties and the microbiome functions are determined by the instances and iterations of their interactions. Thus, the examination of a core composition should also include the specific activities and interactions of the core components, which involve the background conditions. Instead of inferring the activities from the taxonomic composition, the inquiry follows the metabolic pathways and the microbial interactions. This framework better addresses the non-specific nature of the microbiome (i.e., the fact that different microbial compositions can have similar microbiome functions). This non-specific nature of the microbiome is the reason that FMT works without taxa specificity in the case of the treatment for *C. difficile* infection. Therefore, the causal explanation needs an alternative framework that includes the background conditions and the dynamics and heterogeneity of the interactions between microbes, body tissues, and immune cells. Without the consideration of such mutual interactions, all we are left with is a detailed picture of who is present in correlation with the studied trait. What is missing is the specificity of the interactions and background conditions in addition to the taxonomic composition. This way of inquiry looks at the mechanisms of interactions, not only considering the correlation with the presence or absence of bacteria

and is open to include the interactions involving other microbes such as viruses, Archaea, and eukaryotic microbes in the causal explanations.

I have shown that finding the causal entity is not enough without the understanding of the processes of the microbial activity and interactions that involve the background conditions. My suggestion expands on the functional characterization insisting on following the microbial activities in practice, not from inference. This way of inquiry can go along with the authors' suggestion of looking into the causal core in connection with the physiological condition, but with the addition of following the activity and the background conditions as processes of causal interactions. The understanding of who is out there in connection with a disease is essential but not enough. For a better causal explanation, we also need to understand what they (the microbes) do and in what ways they shape their environmental niche. This approach is ecological, which considers the factors of background conditions and interdependence involved in the mutual microbial interactions as causal processes. Thinking about causal explanations that are based on causal processes, not entities, need a new conceptualization. In this conceptualization, the physiological conditions are also viewed as processes, which I believe can be a better fit to address multifactorial traits.

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