25th January 2024

Science  
   
AAAS  
 P.O. Box 96178  
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Dear Editor,

We are herewith submitting our work entitled: "Phenotype switching explains emergence of alternative stable states in a gut microbial community" as a Research Article in Science.

Human gut microbiota occur in different configurations, known as enterotypes. Although differences in host characteristics are frequently cited as the driver behind these alternative community states, they do not explain why transient perturbations are sometimes observed to permanently alter gut microbial community composition. Several hypotheses have been suggested to explain these observations, but no clear experimental evidence in their support has been provided to date.

Here, we show on the example of a synthetic human gut microbial community that phenotype switching is a mechanism that can lead to the emergence of alternative community states. For this, we first explored the life history strategies of three common human gut bacteria using a combination of flow cytometry, metabolomics, and transcriptomics and found a diauxic shift in *Blautia hydrogenotrophica* that alters its interactions with the other species. Next, we represented our findings in a kinetic model, which we parameterized on densely sampled mono- and co-cultures. The model predicted that the community is multi-stable, i.e. that alternative states can occur in the same environmental conditions and depend on the system’s state. We confirmed our predictions with two independent perturbation experiments in vitro, using a state-of-the-art fermentation robot that enabled us to run up to twelve replicate vessels in chemostat mode with a high level of control. The perturbations induced a switch to three alternative community states, which were not observed in the twelve control vessels. This is the first observation of a microbial community shifting to several alternative states after a transient perturbation in controlled conditions.

A key insight resulting from our work is that phenotype switching in response to a transient perturbation alters species interactions, which then induces alternative states. We illustrated in simulations that this mechanism also leads to the emergence of alternative states in larger communities of 50 species. In summary, our work shows that flexible metabolic strategies can explain alternative community states and that multistability is a compelling candidate explanation for the existence of enterotypes.

This experimental evidence of history dependency has implications for the interpretation of alternative microbial community states in relation to host parameters. Given our results, we think that it is important to systematically investigate metabolic strategies of human microorganisms and to accurately represent them in community models to better understand and ultimately to successfully modulate human microbiota.

I confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have read and approved the manuscript.

Kind regards,



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