Relative enrichment of oral bacteria in feces: The Driver vs. Marker hypothesis

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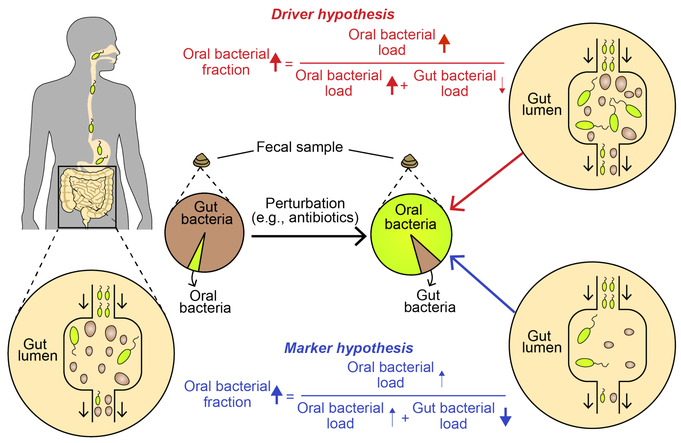
Background: enrichment of oral bacteria in human feces is a biomarker of intestinal disorders

- An average person swallows ~10^{11} of oral bacteria cells per day but oral bacteria remain rare in the healthy gut (<2% fecal DNAs) due to oral-gut barrier (e.g., gastric acids, competition from gut commensals)
- Antibiotics, diet, aging, gut inflammation and other factors can disrupt the oral-gut barrier and cause relative enrichment of oral-associated bacteria in the the feces
- The enrichment has been repeatedly observed in patients with a variety of diseases, including Crohn's diseases, ulcerative colitis, colorectal cancer, liver cirrhosis, etc.

Question: Does the relative enrichment of oral bacteria indicate intestinal bloom of oral pathogens?

- Nearly all studies that found oral bacterial enrichment used compositional data (16S amplicon sequencing or shotgun metagenomics), meaning that the enrichment is relative.
- But relative abundance, by itself, can neither determine if there was an expansion of oral bacteria in absolute numbers nor reveal the true cause of human disorders.
- Simple mathematics leads to two alternate explanations: **In the *driver* hypothesis**, the relative enrichment reflects active expansion of oral bacterial population, which then drives intestinal disorders. By contrast, the relative enrichment is caused by loss of gut commensals **in the *marker* hypothesis**, and it is this bacterial loss that causes the disorders.



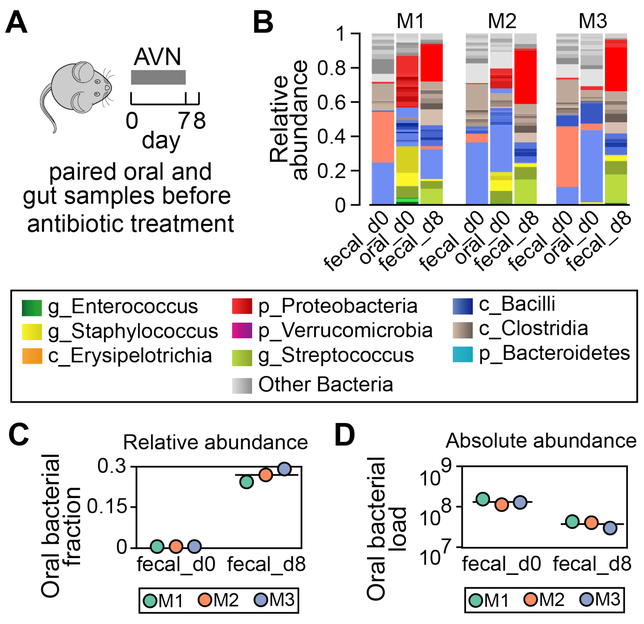
Result I: Mouse experiment with paired oral and gut samples supports the *marker* hypothesis

(**A**) A simple experiment with 3 mice (labeled as M1, M2, M3) treated with an antibiotic cocktail of ampicillin, vancomycin, and neomycin (AVN) for a week. The pre-treatment feces (fecal_d0) and oral swabs (oral_d0) were collected on day 0 and the post-treatment feces (fecal_d8) were collected on day 8.

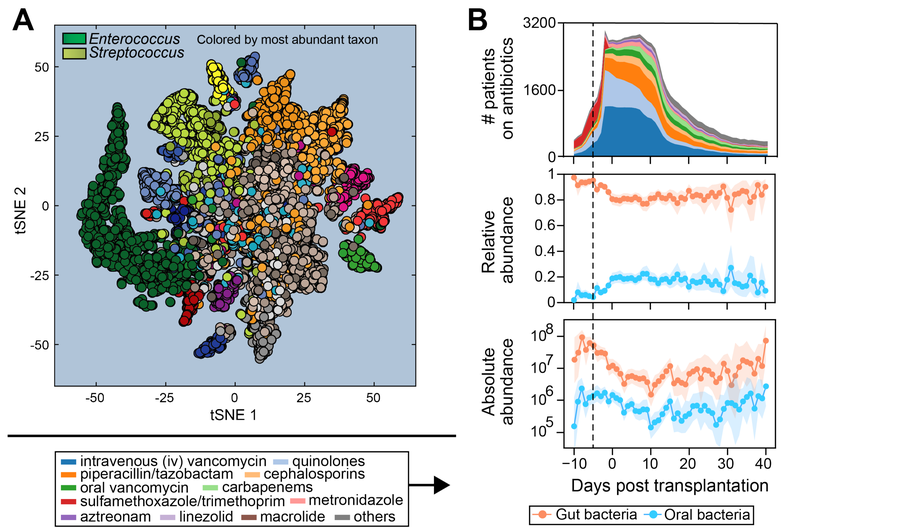
(**B**) Microbiome of all feces and oral swabs prior to antibiotics show very different compositions, but an enrichment of oral bacteria was observed in feces post antibiotics**.**

(**C**) Antibiotic treatment enriched the total relative abundance of oral-typical ASVs (def: > 0.01% across pre-treatment oral samples and ≤ 0.01% on average across pre-treatment fecal samples) in the feces from 0.03% to 27.2% on average across the three mice.

(**D**) However, a quantification of oral bacterial density via qPCR (16S copies per g feces) shows that the relative enrichment of oral bacteria was not due to an increase in their absolute abundance, but driven by a much greater decrease (>5000 fold) in the absolute abundances of gut commensals after antibiotic treatment.



Result II: The *marker* hypothesis is generalizable to cancer patients receiving allogeneic hematopoietic cell transplantation (allo-HCT)

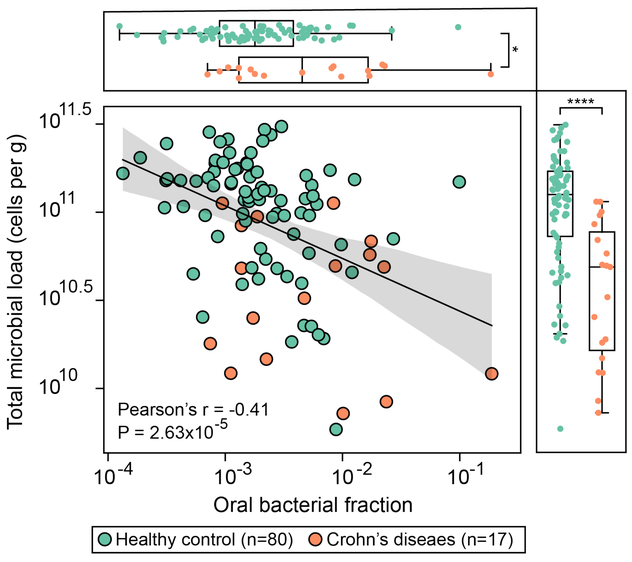


(**A**) Allo-HCT recipients require antibiotics to prophylactically minimize the risk of developing infections before immune system reconstitution or treat infections when they develop, but the antibiotics disrupt their gut microbiome compositions, where *Enterococcus* and *Streptococcus* (typically found in the oral cavity) most frequently dominate the intestinal bacterial communities.

(**B**) The timing of antibacterial antibiotic administration (top) corresponded well to the declined gut bacterial relative (middle) and absolute (bottom) abundances. Since the mean oral bacterial loads fluctuated around a stable average, the increased relative abundance of oral ASVs was mainly driven by the declined gut bacterial load, supporting the *marker* hypothesis.

Result III: The *marker* hypothesis unifies competing microbiome biomarkers of IBD (inflammatory bowel disease)

Clinical studies of IBD have revealed its associations with relative enrichment of oral-associated bacterial species or low absolute values of microbial loads. Since these associations were reported by separate studies, they raise a critical question: are the two IBD biomarkers independent or related? According to the *marker* hypothesis, the two quantities should be dependent and negatively correlated with each other.



Discussion and Conclusions

Our findings support the *marker* hypothesis, i.e., the relative enrichment of oral bacteria in feces indicates loss of gut commensals and it is the bacterial loss that impacts host health. Recognizing that the increased relative abundance of oral bacteria in the gut does not reflect an ectopic bloom will critically inform the interpretation of microbiome compositional data. Since the total fraction of oral bacteria in feces reflects the total bacterial load in the gut, it provides a convenient way to access the size of gut bacterial population and even host status. The robust relationship between the two quantities will lead to new opportunities for diagnosis, prognosis, and treatment of microbiome-related human diseases.