Ecology of plasmids sharing in natural environments

Introduction

Plasmids are extrachromosomal DNA molecules that can replicate independently of the chromosomal DNA. They are typically circular and double-stranded, found predominantly in bacteria but also in archaea and eukaryotic organisms[1]. Plasmids carry genes that can provide various advantages to their host organisms, such as antibiotic resistance, virulence factors, and metabolic capabilities that are not encoded by the chromosomal DNA[2,3]. This makes plasmids crucial for horizontal gene transfer, which is a significant driver of genetic diversity and evolution in microbial populations.

In natural environments, plasmids play a pivotal role in microbial ecology by facilitating the adaptation and survival of microbial communities. They enable bacteria to rapidly acquire and disseminate beneficial traits in response to environmental pressures, such as changes in nutrient availability, presence of toxins, or competitive interactions with other microorganisms[4,5]. This capability is particularly important in dynamic ecosystems like the rumen, where microbial communities must constantly adjust to the changing diet and conditions of the host animal. The ecological significance of plasmids extends beyond individual benefits, as they contribute to the overall functionality and stability of microbial ecosystems by promoting genetic connectivity and diversity[4].

Plasmid segments sharing, or the exchange of plasmid DNA fragments between different microbial hosts, is a critical mechanism in the horizontal gene transfer process. This phenomenon enhances the genetic repertoire of microbial communities and facilitates the spread of adaptive traits[6]. In the context of the rumen microbiome, plasmid segments sharing can influence the assembly and dynamics of microbial populations, affecting the efficiency of nutrient digestion and overall health of the host. Understanding the patterns and drivers of plasmid

segments sharing is essential for comprehending how microbial communities evolve and function in complex environments.

Methods

Experimental Design

This study involved a longitudinal analysis of the rumen microbiome from birth up to three years old, using 12 metagenomic samples collected over different time points. These samples were processed to extract DNA, followed by metagenomic sequencing.

Network Construction

At first, microbial genomes and plasmids were assembled from the metagenomic samples. Assembled plasmids were then analyzed to detect shared segments between different microbial hosts. The detection involved aligning plasmid sequences to identify common stretches of DNA, indicative of segment sharing.

A multi-layer network was constructed to represent the plasmid segments sharing among microbial hosts over time. In the network nodes represent microbial hosts, edges represent the sharing of plasmid segments between hosts, and weights on the edges indicate the number of shared plasmid segments. Each layer of the network corresponds to a specific time point, capturing the dynamics of plasmid segments sharing across the 12 time points.

Network Analyses

To understand the importance and influence of each microbial host in the plasmid sharing network, eigenvector centrality (EC) was calculated for every node in two different contexts: multi-layer and mono-layer. Multi-layer EC considered the full network, integrating all time points. EC in this context measures the influence of a node not only based on its direct connections but also considering the influence of its neighbors across all layers. This comprehensive view is crucial for identifying microbial hosts that consistently play a central role in plasmid sharing throughout the entire study period. Mono-layer EC focused on each individual time point,

treating each layer separately. In this context, EC helps to identify the most influential nodes at specific time points, highlighting temporal variations in the network's structure and the dynamic roles of microbial hosts in plasmid sharing.

To examine modules of information flow in which groups of microbial hosts are more densely connected to each other than to nodes from other modules, modularity analysis was done using Infomap[7]. Examining modules of information flow is important because it highlights functional groups within the microbial community that might be cooperating or competing more closely with each other.

Results

Eigenvector centrality suggests three different roles based on plasmid segments sharing in microbial hosts

The eigenvector centrality (EC) analysis, comparing the multi-layer and mono-layer contexts, revealed several distinct patterns among the microbial hosts (**Figure 1A**). A group of microbial hosts that exhibited high importance in individual time-points (mono-layer) but had zero importance when considering the entire network (multi-layer). This suggests that these hosts play crucial roles at specific time-points, possibly due to transient conditions or short-term interactions that are significant only within particular times. These hosts might be opportunistic species that exploit temporary niches or resources, contributing to dynamic shifts in the microbial community structure and introducing short-term genetic variations that allow the community to quickly respond to transient challenges or opportunities. A second group of microbial hosts had high importance across the full network (multi-layer) but were not significant in any individual time-point (mono-layer). These hosts may act as stable, central players in the plasmid sharing network over the long term, facilitating consistent genetic exchange across different stages of the microbiome’s development. Their persistent influence suggests they might be keystone species or central hubs that maintain the overall connectivity and resilience of the microbial community, ensuring stable gene flow and ecological balance. A third group of microbial hosts were important in both the mono-layer

and multi-layer contexts. These hosts are likely integral to both short-term and long-term dynamics of plasmid sharing. They could be pivotal species that are consistently active in genetic exchange, regardless of the temporal context, serving as critical connectors that integrate transient interactions with the broader, more stable network structure. These hosts might represent highly adaptable species that can thrive under varying conditions, contributing to both immediate responses and sustained functionality of the microbiome. Their adaptability and centrality makes them key players in integrating immediate ecological shifts with the ongoing evolutionary processes within the rumen microbiome.

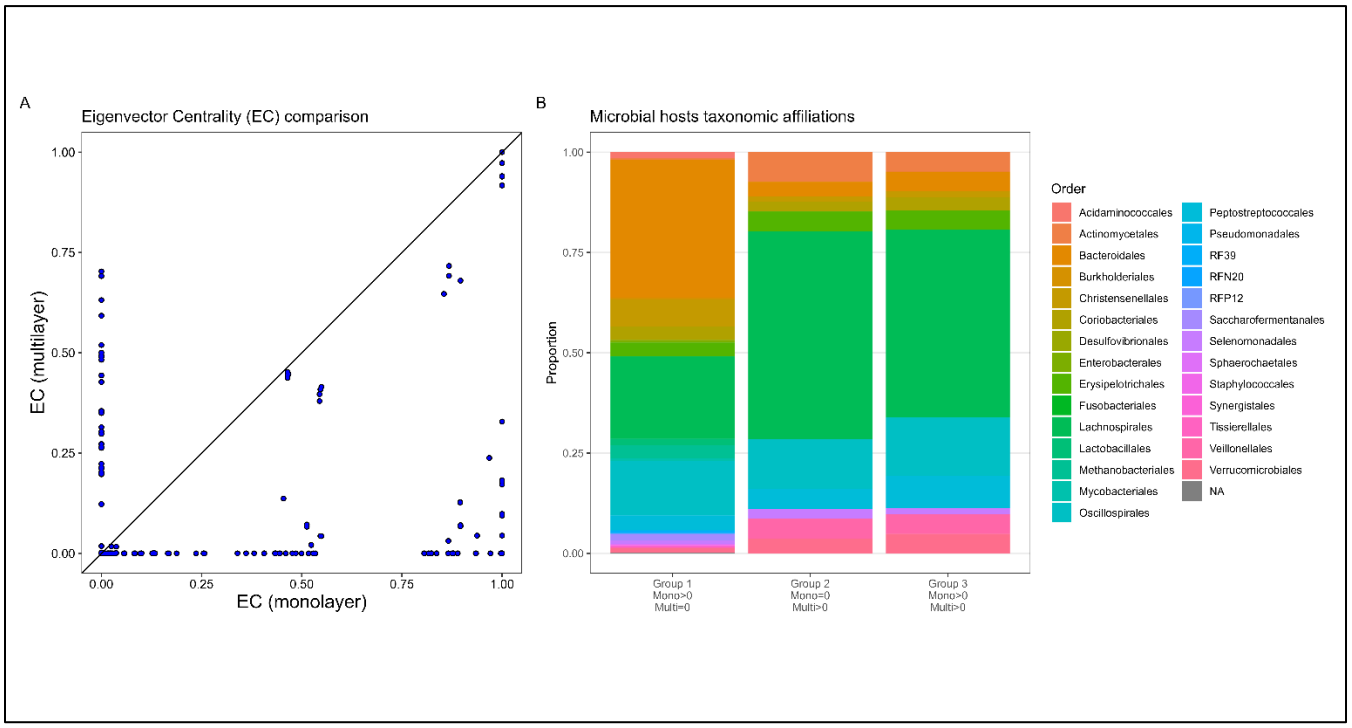

Figure 1 | (**A**) Eigen-vector centrality comparison of microbial hosts between mono-layer context (x-axis) and multi-layer context (y-axis); (**B**) Microbial hosts taxonomic affiliation based on their group defined by the EC comparison: (1) high mono EC and low multi EC, (2) low mono EC and high multi EC, (3) high mono EC and high multi EC.

Taxonomic affiliations of the microbial hosts revealed differences in compositions between the different groups (**Figure 1B**). Group 1, characterized by high mono-layer EC and low multi-layer EC, was rich with *Bacteroidota* and uniquely included *Proteobacteria* and *Methanobacteriota*. This suggests that *Bacteroidota* phyla,

known for their role in degrading complex polysaccharides, might be crucial for rapid adaptation to dietary changes, thus becoming important in specific time-points. The presence of *Proteobacteria* and *Methanobacteriota*, which are involved in diverse metabolic processes including methane production, indicates that these taxa might exploit transient ecological niches or respond to short-term environmental fluctuations. Groups 2 and 3, which included microbial hosts with high multi-layer EC, were dominated by *Firmicutes*. *Firmicutes* are known for their robust and versatile metabolic capabilities, contributing to the stability and resilience of the rumen microbiome. Their prevalence in these groups supports the hypothesis that they act as stable connectors (Group 2) or adaptive integrators (Group 3), ensuring consistent genetic exchange and maintaining the overall structure and function of the microbial community across different time points.

Persistent and transient modules accounts for the majority of the information flow

Modularity analysis on the multi-layer network identified over 100 modules of microbial hosts that were more densely connected within themselves than with nodes outside the module. Based on Figure 1 results, I hypothesized that both persistent and transient modules will take place under plasmid segments sharing during the assembly process of the rumen microbiome. The persistence throughout the entire study period suggests for microbial hosts that form the core of the rumen microbiome, maintaining essential functions and stability in the microbial community. These hosts might be crucial for maintaining the baseline ecological balance and facilitating continuous genetic exchange, ensuring the microbiome's resilience and adaptability. On the other hand, transient modules likely represent microbial hosts that respond to specific environmental cues, such as changes in diet. These hosts may introduce new genetic material or adapt their metabolic functions to exploit new resources or conditions, thereby contributing to the dynamic adaptation of the microbiome. Appearance and disappearance of such modules will highlight the microbiome's ability to quickly adjust to environmental fluctuations, enhancing its overall adaptability and functional diversity.

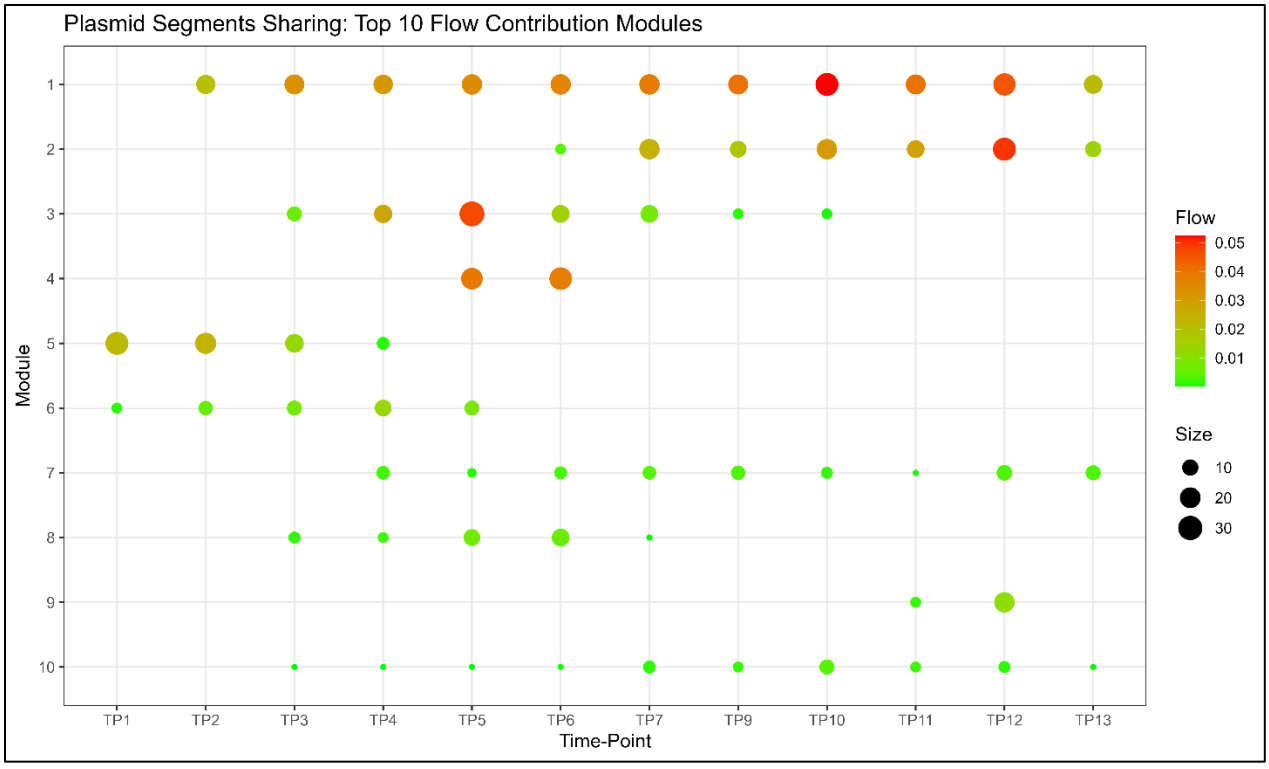

Figure 2 | Plasmid segments sharing multi-layer network modularity analysis, showing the top 10 flow contribution modules. Time-points are shown on the x-axis, module assignment is shown on the y-axis, size of points corresponds to the number of microbial hosts belonging to the module in a given time-point and the color represents the total flow of information (green-low, red-high).

Focusing on the top 10 modules, which accounted for over 90% of the overall flow of information in the network, revealed significant insights into the plasmid-sharing dynamics in the rumen microbiome (**Figure 2**). These top 10 modules are crucial for understanding the network as they encapsulate the majority of the information flow. Among these, module 1 was particularly prominent, holding 40% of the total flow and persisting across all layers. This indicates a stable and central group of microbial hosts that consistently play a pivotal role in the plasmid-sharing network. In contrast, some modules were transient, appearing and disappearing at different stages (e.g., module 2 (TP6-TP13), module 3 (TP3-TP10), module 4 (TP5-TP6), modules 5,6 (TP1-TP5)). These transient modules may reflect dynamic shifts in the microbiome, possibly in response to environmental changes or specific events in the cow's life. Notably, certain modules corresponded with changes in the

animal's diet, emerging after dietary shifts, suggesting a strong link between environmental conditions and the assembly process of the rumen microbiome. A particular noticeable change appears around TP5-TP6, coinciding with the animal host’s transition to a fiber-based diet. During this period, several notable shifts were observed: module 2 appeared at TP6, module 3's flow increased at TP5, module 4 existed only between TP5-TP6, and modules 5 and 6 disappeared around TP5. These changes suggest a strong influence of dietary transition on the microbial community structure and plasmid-sharing dynamics.

Conclusions

The study of plasmid segments sharing within the rumen microbiome revealed a complex interplay of microbial interactions driven by genetic exchange. Centrality analysis showed that certain microbial hosts are pivotal for plasmid sharing at specific times, while others maintain consistent influence across the entire network. This underscores the importance of both transient and stable interactions in the microbial community's genetic landscape. The modularity analysis further highlighted that a few key modules dominate the network's information flow, indicating concentrated hubs of plasmid sharing. The significant shifts around TP5-TP6, corresponding to the dietary transition to fibers, underscores how environmental changes can reshape these genetic exchange networks.

Several follow-up questions emerge from these findings: How do specific plasmid segments contribute to the functional capabilities of different microbial hosts? How does the plasmid-sharing network influence the overall stability and resilience of the rumen microbiome? Investigating these questions could provide deeper insights into the ecological and evolutionary significance of plasmid segment sharing in natural microbial communities.

References

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