GENOME WATCH

Welcome to the plasmidome

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This month's Genome Watch reviews a recent paper that used a metagenomics approach to characterize the plasmid content of bovine rumen samples.

Plasmids are self-replicating, extrachromosomal mobile genetic elements that can be transferred between bacterial hosts. As well as encoding the apparatus required for self-replication, they can carry a diverse array of accessory genes, including some with important ramifications for human health, such as antibiotic resistance genes. As a result, plasmids are important mediators of lateral gene transfer (LGT), a process that significantly influences bacterial activity and evolution.

The bovine rumen is home to a diverse and abundant microbiota (comprising $\sim 10^{10}$ cells per ml of contents) that converts otherwise indigestible plant biomass into compounds that can be used by the bovine host. The high bacterial load within the rumen may facilitate widespread LGT, but research into rumen plasmids has been hampered by the fact that most rumen microorganisms remain uncultured.

To circumvent this problem, Kav *et al.*¹ recently reported a metagenomics approach to study the 'plasmidome' (that is, the overall plasmid content in a given environment). The authors purified plasmid-derived DNA from 16 cow rumens by digesting chromosomal DNA using a plasmid-safe DNase (which preferentially degrades linear DNA) and then amplifying circular DNA using the DNA polymerase from phage $\phi 29$. The amplified plasmid DNA was sequenced using the Illumina platform, generating 34 million reads, 36.8% of which were assembled into 5,771 contigs with a mean length of 469 bp. The fragmented

nature of this assembly indicates that even the impressive sequencing depth generated in this study was insufficient to fully characterize the rumen plasmidome, which is evidently highly diverse.

The authors created phylogenetic assignments for their plasmid contigs and then compared these results to a 454 sequencing-based 16S ribosomal RNA gene survey of the microbiota in their samples. Most sequences from both data sets were assigned to the phyla Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteria, which was expected as these phyla are typically dominant within mammalian intestines. However, there were differences in the proportional abundances between the two data sets, with more Proteobacteria assignments in the plasmidome than in the microbiota. The authors speculate that this might be because Proteobacteria members harbour more plasmids than bacteria from other phyla in the rumen, although they concede that it could also result from technical biases, such as those generated during the DNA amplification step.

Plasmids can be mosaic in structure, incorporating DNA from various sources, so in cases in which more than one protein-coding sequence was present on a contig the authors attempted to classify each coding sequence separately. Potentially mosaic plasmid sequences were detected, 14.3% of which showed evidence of cross-phylum mosaicism. This suggests that, although LGT is most common among phylogenetically related species, gene mobility between different phyla is still fairly common in microbially rich environments such as the rumen.

The authors then compared the putative coding potential of the plasmidome with those of two previously published rumen metagenomes^{2,3}

and to databases of previously reported plasmid sequences. This functional comparison confirmed that the rumen plasmidome encodes more plasmid-specific functions and virulence factors than were detected in the rumen metagenome data sets. However, it also showed that the rumen plasmidome has a distinct functional gene profile from the overall plasmid databases, with more putative coding sequences for features such as carbohydrate and protein metabolism and cell wall or capsular factors. Many of these putative plasmidome-enriched functions are likely to enhance microbial host survival within the rumen.

These findings indicate that plasmid content, as well as overall bacterial community composition, may be selected for by the ecological niche. In support of this conclusion, the authors showed that many of their putative plasmid sequences had been isolated previously from bovine samples collected from various locations and at different time points. This conclusion will need to be validated by studying plasmidomes from other environments but is consistent with previous work suggesting that viruses from different environments carry discriminatory functional gene profiles⁴.

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Competing interests statement

The author declares no competing financial interests.

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