MICROBIOLOGY
SOCIETY

15–16 September 2022

Edgbaston Park Hotel and Conference Centre,
Birmingham, UK

Poster Abstract Book

microbiologysociety.org/GenomesMicrobiomes22
#GenomesMicrobiome22

P1

Identification of viral transcripts in RNA-seq datasets from bees, wasps, mites and ants.

Katherine Brown, Andrew Firth

University of Cambridge, Cambridge, United Kingdom

Abstract

Many honey bee colonies suffer large losses due to colony collapse disorder. This phenomenon has led to widespread efforts in sequencing honey bee pathogens, including RNA viruses. However, honey bees coexist with a number of other arthropods, whose viruses are less thoroughly characterised. Many viruses currently classified as honey bee pathogens may therefore have a much wider host range. In particular, ants often coexist with bees and the two groups have previously been shown to exchange viruses. Parasitism by Varroa mites, known to act as effective vectors for a number of RNA viruses, is also almost ubiquitous amongst honey bees, but relatively little is known about viruses endemic to mites.

We have previously demonstrated that it is possible to detect and characterise viral RNA in publicly available RNA-seq datasets. There are over 3,000 such datasets for diverse Hymenoptera and mite species. We have therefore developed a computational pipeline to identify viral transcripts in these datasets. This pipeline performs quality control, filters and assembles reads into transcripts and detects the presence of regions with homology to known RNA viruses. Over 20,000 putative viral fragments have been identified in these datasets, forming almost 700 clusters. Phylogenetic analysis of these clusters has revealed both previously unknown viruses and known viruses in unexpected hosts. We have also developed a bioinformatics tool, CIAlign, used to clean and visualise multiple sequence alignments, to mitigate issues encountered while developing this pipeline.

P2

Bacterial Interactions within the Upper Respiratory Tract Microbiota

Sarah Almuhayya[1,2], Gavin Humphreys[1], Andrew Mcbain[1]

[1]The University of Manchester, Manchester, United Kingdom. [2]King Saud University, Riyadh, Saudi Arabia

Abstract

Background. The microbiome of the upper respiratory tract (URT) has received less research attention than other body sites. This study aims to investigate the microbial ecology of the human URT with a focus on antagonism between the corynebacteria and staphylococci.

Methods. Mucosal swabs were collected from the anterior nares and nasal turbinates of 20 healthy adult subjects. Genomic DNA amplification targeting the (V4) of the 16Sr RNA gene was conducted and analysed using QIIME. Nasal swab isolates were cultured and identified using near full-length sequencing of the 16S rRNA gene. Isolates identified as corynebacteria or staphylococci were typed using (rep-PCR). Antagonism was determined using an agar-based inhibition assay.

Results. Four major bacterial phyla (*Actinobacteria, Bacteroidetes, Firmicutes,* and *Proteobacteria*) were identified from all volunteers. The typing of cultured staphylococci and corynebacteria suggested that intra-individual strain diversity was limited. Analysis of generated nasal microbiota profiles suggested an inverse correlation in terms of relative abundance between staphylococci and corynebacteria. Despite the apparent antagonism between these genera, it was limited when investigated on agar. Of 1000 pairwise interactions, observable zones of inhibition were only reported between a single strain of *C.pseudodiphtheriticum* and *S.aureus*. Imaging under EM revealed this effect to be bactericidal with clear lytic effects on staphylococcal cell morphology.

Conclusion. Nasal microbiota is complex, but culturable staphylococci and corynebacteria were limited in terms of clone type. Analysis of generated nasal microbiota profiles suggested an inverse correlation in terms of relative abundance between these genera suggesting an antagonism or competition between these taxonomic groups.

P3

Using metagenomics to study the resident microbiome of food production settings, with a focus on *Listeria monocytogenes*.

Maria Diaz[1], Heather Aird[2], Ana Victoria Gutierrez[1], Oleksii Omelchenko[1], Nicolle Som[1], Jim McLauchlin[3]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]UK Health Security Agency, York, United Kingdom. [3]UK Health Security Agency, London, United Kingdom

Abstract

Understanding the resident microbiota of food industry environments can help develop specific strategies to prevent contamination of food products. Metagenomic analysis is a rapid and powerful approach to study these microbial populations. However, specific techniques are needed to increase sensitivity when aiming to detect certain pathogens, such as *Listeria monocytogenes*, that may be present at very low abundance in these complex communities.

As a proof of concept, environmental swab samples were collected from 15 non-food contact locations in a RTE-food processing setting over six months. A process to optimise nucleic acid extraction from swabs was undertaken. Microbial diversity of the resident microbiome was analysed by short-read shotgun sequencing of DNA extracted directly from the environmental swabs. Metagenomic detection of *Listeria* spp. required culture-based enrichments and was performed using short and long-read sequencing, in comparison with routine methods for microbiological detection of *Listeria* spp.

In this study, we evaluated the microbial structure of the residential microbiota over time, which was dominated by the genera *Pseudomonas* and *Sphingomonas*. *Listeria* spp. could not be detected in the environmental metagenomes directly obtained from swabs. Alternatively, *L. monocytogenes* and other *Listeria* species could be reliably detected in these enriched metagenomes. In addition, complete, closed *Listeria* genomes were obtained from culture-based enrichments when long-read nanopore sequencing was applied. Our results demonstrate the challenges of using metagenomics to study low abundance microorganisms and show the potential of using a combination of culture and sequencing approaches to improve the detection and identification of key bacterial taxa in environmental samples.

P4

The next-generation tools for risk assessment and precision food safety: use of shotgun metagenomics sequencing for the characterisation of milk resistome and investigation of MAGs

Guerrino Macori[1], Selene Rubiola[2], Francesco Chiesa[2], Tiziana Civera[2], Séamus Fanning[1]

[1]University College Dublin, Centre for Food Safety, Dublin, Ireland. [2]Department of Veterinary Sciences, University of Turin, Turin, Italy

Abstract

Understanding the distribution and genomic dynamics of antimicrobial resistance (AMR) in complex food matrices such as milk has great relevance for protecting consumers and maintaining high food safety standards. Herein, the resistome of ten dairy farms was investigated through a shotgun metagenomic sequencing approach, sampling in-line milk filters. The application of both reads-based and assembly-based approaches, facilitated the comprehensive characterization of the resistome, while also allowing the construction of complete metagenome-assembled genomes (MAGs) and the investigation of mobile genetic elements. Notably, most of the species harbouring AMR genes were predicted to be Gram-negative genera, including *Enterobacter, Acinetobacter, Escherichia,* and *Pseudomonas,* pointing out the role of these bacteria as reservoirs of AMR genes. Eight high-quality MAGs, whose genome sizes varied from 1.3 to 4.2 Mb were identified filtering 1,500 bp or longer scaffolds. One MAG belonged to the phylum Gammaproteobacteria, while seven MAGs were identified at the genus or family level, including one *Psychrobacter sp*., two *Acinetobacter spp*., one *Lactococcus sp*. and one member each of the *Moraxellaceae, Enterobacteriaceae* and *Weeksellaceae* family. 22 AMR genes were identified in the MAGs, corresponding to 14 AMR mechanisms and 6 AMR classes, including multidrug, β-lactam, aminoglycoside, tetracycline, AMPs and fosfomycin classes. According to this study, raw milk can be considered a source of AMR bacteria and translating these findings as risk assessment outputs heralds the next generation of food safety controls which, in future would include also the evaluation of high-quality MAGs as a tool for precision food-safety.

P5

Exploration of Bacteroides phage diversity and characterization of a novel Bacteroides fragilis phage family

Fiona Newberry

Nottingham Trent University, Nottingham, United Kingdom. Quadram Institute Bioscience, Norwich, United Kingdom. University of East Anglia, Norwich, United Kingdom

Abstract

The aim of this study was to investigate the genetic relationship of B.fragilis phage within the context of currently known phage and metagenome-assembled Bacteroides phage.

All complete Bacteroides phage from NCBI Virus and Bacteroides-predicted host phage from IMGVR and the GPD were downloaded, resulting in a total of 2,639 phage sequences. Ten representative crAss-like phage genomes were also included in analyses. A gene-gene network was generated to examine the taxonomic relationship of the manually curated Bacteroides phage dataset to known phage. A total of 465 viral clusters (VCs) and 916 sub-VCs were identified, with 97 VCs composed exclusively of Bacteroides phage highlighting the unexplored Bacteroides phage diversity. Five known phage genomes interacted with the Bacteroides cluster and may represent the closest known viral relatives. All known B. fragilis phage were grouped within the same VC. Nucleotide-based intergenomic similarity analysis revealed this VC represents a novel phage family, with 5 distinct genera and 37 species. A total of 21 proteins were conserved across the family; however, structural proteins were not universally conserved across the family and showed genera-level conservation.

This study used metagenome-based phage discovery approaches to identify a novel B. fragilis phage family unrelated to crAssphage, and revealed the unexplored diversity of Bacteroides phage. Despite the importance of Bacteroides within the human gut microbiota, only 45 Bacteroides phage have been fully characterised to date with the majority isolated using Bacteroides thetaiotaomicron.

P6

Shotgun metagenomics vs. 16S rRNA amplicon sequencing: which tells us more about how the equine gut microbiota develops in early age.

Joy Leng[1], Rachel Gilroy[2], Ruth O'Flaherty[1], Robert Mansergh[3,1], Rachael Cooke[1], Richard Ellis[4], Falko Steinbach[1,4], Mark Pallen[2], Roberto La Ragione[1], Chris Proudman[1]

[1]The University of Surrey, Guildford, United Kingdom. [2]The Quadram Institute, Norwich, United Kingdom. [3]University College London, London, United Kingdom. [4]Animal and Plant Health Agency, Weybridge, United Kingdom

Abstract

Gut microbial communities in the horse colon are associated with many key physiological functions including digestion and immune system development. However many of these bacteria are unculturable and are often not represented in reference databases used to analyse 16S rRNA amplicon sequences. To obtain a better insight into how the gut microbiota of horses develops, we subjected faecal extracts to 16S rRNA amplicon and shotgun metagenomic sequencing. Faecal samples were collected from ten Thoroughbred racehorse foals in the first year of their lives, at 2, 8 14, 28, 60, 90, 180, 272 and 365 days of age. Sequence files from 16S rRNA amplicons were processed and analysed using QIIME2. Sequences produced using Illumina shotgun sequencing were individually assembled into contigs using MegaHIT before being binned to produce more than 1,800 metagenome assembled genomes (MAGs) using CONCOCT, MaxBIN and MetaBAT2. We identified 857 metagenomic species clusters (95 % ANI) with only 24 % representing known species. Both sequencing approaches showed low taxonomic diversity in early samples which increased until the horses were two months old. Using hierarchical and Partitioning Around Medoids (PAM) clustering algorithms, we identified three compositional clusters within our equine sequences. Differential abundance analysis identified the enrichment of *Enterobacteriaceae* species in young foals at 2 days of age, moving towards enrichment of *Bacteroidaceae* species alongside multiple novel species by four month of age onwards. This study highlights the richness of the equine gut microbiome and its role as a reservoir for new bacterial species.

P7

Metagenomics to Trace Pathogens and AMR in Poultry Distribution Networks in India, Sri Lanka, Bangladesh, and Vietnam

Melanie Hay[1], Damer Blake[1], Ankit Hinsu[2], Prakash Koringa[2], Fiona Tomley[1]

[1]Royal Veterinary College, Potters Bar, United Kingdom. [2]Anand Agricultural University, Anand, India

Abstract

Antimicrobial resistance (AMR) poses an enormous threat to global public health. Unfortunately, antimicrobial resistance genes (ARGs) do not obey political, geographical or species borders and the propensity of new ARGs to develop and disseminate among humans, animals, and the environment necessitates the use of a One Health approach for its study and management. The poultry industry in Asia is at high risk for the emergence of AMR because the use of antibiotics as growth promoters on poultry farms makes them likely reservoirs of ARGs. Bacteria acquire and spread AMR via the horizontal gene transfer (HGT) of ARGs located on mobile genetic elements such as plasmids. Identifying the bacterial hosts of these plasmids alerts us when AMR has arisen in human pathogens as well as when multi-drug resistance is occurring either on the same plasmid or on different plasmids in the same host. Additionally, information about where these genes are in the plasmid or chromosome can tell us how ARGs may be regulated and how easily they might spread. In this study, we plan to examine the spread of AMR from poultry farms to markets to humans using a combination of Hi-C proximity ligation with Illumina shotgun sequencing and long-read sequencing with Oxford Nanopore to reconstruct MAGs and plasmids. The results will help highlight potential public health risks in the Asian poultry industry.

P8

Selective nanopore sequencing increases accuracy of *Campylobacter* assembly from stool metagenomes

Bilal Djeghout[1], Thanh Le-Viet[1], Rhiannon Evans[1], David Baker[1], Ngozi Elumogo[1,2], Andrew Page[1], John Wain[1,3], Nicol Janecko[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Eastern Pathology Alliance, Norfolk and Norwich University Hospital, Norwich, United Kingdom. [3]Faculty of Medicine, University of East Anglia, Norwich, United Kingdom

Abstract

Full genome assembly of low abundance gastrointestinal pathogens, such as *Campylobacter* remains a sequencing challenge in microbiomes. As public health laboratories move from culture methods towards PCR, a genomic data gap for *Campylobacter* genome characterisation from stool is formed.

The aim was to determine the capabilities of selective Nanopore sequencing in assembling full *Campylobacter* genomes from a defined mock community and natural stool microbiomes.

A mock community and host-depleted DNA extracted from diarrheal stool were spiked with *Campylobacter jejuni* (2% of total DNA); duplicates were sequenced on the MinION sequencer using selective mode to enrich sequencing of *Campylobacter* DNA strands and Standard mode sequencing, in parallel. Metagenome assembled genomes (MAGs) were reconstructed with a standard bioinformatics workflow: filtering (filtlong), trimming adapters (porechop), de-novo assembly (Flye), polishing (medaka), frame-shift correction (proovframe), completeness validation (BUSCO), genome annotation (PROKKA).

Selective sequencing resulted in 2.5-fold sequence depth increase of *C. jejuni* in the mock community and accounted for 47% of the total reads and 9.27% in standard mode. Complete assemblies were achieved for both; *C. jejuni* genome completeness was higher using selective sequencing mode. In diarrheal stool, selective sequencing resulted in 153X coverage of *C. jejuni* and 11.86% of total sequenced DNA; standard sequencing resulted in 248X coverage and 6.42% sequenced DNA. Once assembled, *C. jejuni* from selective sequencing had higher completeness (93%) than standard sequencing (92.20%).

Selective nanopore sequencing enhanced the proportion and precision of *Campylobacter* genome assembly for further genome characterisation, adding value to a specialised sample preparation, sequencing and informatic approach.

P9

Linking antimicrobial resistance genes to their bacterial hosts in the human gut microbiome using Hi-C

Gregory McCallum[1], Amanda Rossiter[1], Sarah Kuehne[2], Willem van Schaik[1]

[1]Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom.
[2]The School of Dentistry, University of Birmingham, Birmingham, United Kingdom

Abstract

The complex microbial ecosystem inhabiting the human gut, termed the gut microbiome, can act as a reservoir for antimicrobial resistance genes (ARGs), collectively known as the gut resistome. Various methods have been developed to study the resistome, including using proximity ligation techniques, such as Hi-C, to link bacterial genes to phylogenetic markers, allowing ARGs to be linked to their bacterial host.

Here, we implemented Hi-C to investigate the bacterial hosts of ARGs in 4 human faecal samples. Using binning techniques on metagenomic assemblies from these samples, combined with Hi-C data, we were able to link 87 ARGs to their hosts across the 4 samples, out of a total of 119 ARGs identified by shotgun sequencing. ARGs carried on plasmids in an *Acinetobacter pittii* strain that was used as a spike-in were correctly linked to their host in all samples. We found that Hi-C was able to link ARGs to multiple contigs in each metagenomic assembly, and the main limiting factor in identifying the bacterial hosts of the ARGs was the success of the binning process and the ability to taxonomically classify the bins.

Following Hi-C analysis, the hosts of several ARGs were successfully cultured and whole genome sequenced. These sequencing data provided genomic context for the ARGs, and offered insights into the limitations of using Hi-C to link ARGs to their host in complex metagenomic samples. Overall, our data highlight the complementarity of Hi-C and culture-based approaches to fully characterise the gut resistome.

P10

MINUUR: Microbial Insights Using Unmapped Reads – Application in *Aedes aegypti*

Aidan Foo, Louise Cerdeira, Grant Hughes, Eva Heinz

Liverpool School of Topical Medicine, Liverpool, United Kingdom

Abstract

The mosquito microbiome influences multiple life-history traits, including vectorial capacity. The application of whole genome shotgun sequencing is commonly used in mosquito genomics; meaning non-mosquito sequence data (we refer to these as unmapped reads) are a source to identify mosquito microbiome members using metagenomic approaches. We developed a reproducible snakemake pipeline to provide **M**icrobial **IN**sights **U**sing **U**nmapped **R**eads (**MINUUR**) from short read whole genome sequencing data. The pipeline separates host and non-host reads, and characterises the latter using i) read classification ii) functional read profiling and iii) de novo metagenome assembly, binning and quality assurance. We applied this pipeline to a previous study which produced mosquito (*Aedes aegypti*) whole genome sequencing data associated to high and low dengue blocking mosquito populations - publicly available on the European Nucleotide Archive. We classified microbial taxa to genus and species level, identifying dominant *Ae*. *aegypti* symbionts within *Proteobacteria* and *Bacteroides* and show the symbiont *Pedobacter* is uniquely present in high dengue-blocking mosquito samples, congruent with a previous study. Using the search term "siderophore", we identified 17 siderophore related genes associated to seven bacteria - the most common was the *TonB* dependent siderophore receptor. Finally, we assembled 19 high-quality draft MAGs which were classified using the GTDB and will be used for further analysis. The result of this work is a metagenomic analysis of non-host reads from *Ae. aegypti* whole genome sequencing data within a reproducible pipeline.

P11

A waxy treasure trove of genomic data - Highlighting the potential of brood and beeswax for the genomic surveillance of American Foulbrood

Guillermo Rangel-Pineros[1], Max Ramsøe[1], Tuuli Kasso[1], Thomas Sicheritz-Pontén[1], Per Kryger[2], Matthew Collins[1,3], Christian Carøe[1]

[1]The Globe Institute - University of Copenhagen, Copenhagen, Denmark. [2]Aarhus Universitet, Aarhus, Denmark. [3]McDonald Institute for Archaeological Research, Cambridge, United Kingdom

Abstract

Genomic surveillance of American Foulbrood (AFB), the most devastating disease of honeybees, is typically achieved via the isolation, culturing and genomic sequencing of Paenibacillus larvae strains infecting honeybee larvae. Here, we report on the recovery of 31 nearly-complete P. larvae genomes from DNA contained in infected hives sampled over 30 years. Brood and beeswax samples from hives showing AFB symptoms were collected from farms around Denmark. Shotgun PE libraries were prepared from extracted DNA and sequenced with Illumina NovaSeq 6000. Sequencing data were processed to assemble and bin putative P. larvae genomes, which were employed to generate a core genome-based phylogenomic tree including 500 publicly-available P. larvae genomes. High-quality MAGs classified as Paenibacillaceae based on single-copy taxonomic markers were obtained for 82% of the processed samples. The recovered genomes were more closely related to reference P. larvae strains than to reference sequences from other Paenibacillus species, according to all-vs-all average nucleotide identity values. The phylogenomic tree demonstrated that our set of Danish P. larvae MAGs spans nearly all the identified major clades, including genomes closely related to isolates previously recovered in Europe, Asia and Oceania. Our results demonstrate that recovering high-quality P. larvae genomes directly from brood and beeswax samples without the need for culturing is feasible and useful for studying the diversity of circulating strains. These observations suggest that AFB outbreaks could potentially be monitored in a more efficient way through the analysis of DNA extracted directly from infected specimens.

P12

The effect of dietary bioactives on the gut microbiome diversity (DIME) – A pilot study

Duncan Ng[1], Federico Bernuzzi[1], Jennifer Ahn-Jarvis[1], Maja Omieljaniuk[1], George Saava[1], Paul Finglas[1], Falk Hildebrand[1,2], Maria Traka[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Earlham Institute, Norwich, United Kingdom

Abstract

The gut microbiota is crucial for the determination of host health. Its functions vary from producing essential nutrients for the host to preventing the colonisation of pathogens. Changes in the diet have been shown to modulate the gut microbiota. Phytochemicals (non-nutrient food components) found in fruit and vegetables can modulate metabolic processes and promote better health. However, the interaction between phytochemicals and gut microbiota is poorly understood.

To address this, we conducted a randomised 2x2 crossover study to estimate the effect of diets rich in dietary bioactives on the gut microbial diversity and markers of metabolic health in 20 healthy participants. Participants consumed a diet high in bioactive-rich food (containing polyphenols, Sulphur (S)-metabolites, and carotenoids) and a diet low in bioactive-rich food for 2 weeks, with a washout period of 4 weeks in between. Extensive food diaries are collected and analysed to ensure compliance with the diet. We will collect 5 faecal samples each (before & after interventions, and between the washout period) with 100 samples. These are subjected to shotgun metagenomics and untargeted metabolomics. We expect to observe a change in the gut microbiota in response to the dietary intervention, particularly in the high bioactive diet where we hypothesise an increase in microbial diversity. The metabolomic data will allow us to identify changes in the metabolites as a result of the shift in the gut microbiome.

P13

Developing novel techniques for molecular Microbial Source Tracking of Welsh bathing waters.

Aliyah Debbonaire, Dr Arwyn Edwards

Aberystwyth University, Aberystwyth, United Kingdom

Abstract

The enumeration of viable faecal indicator organisms (FIOs) is essential to monitor bathing waters to maintain public health. Current methods to monitor FIOs in Welsh bathing waters are culture-based and rely on detection thresholds of FIOs. The development of molecular methods for Microbial Source Tracking-(MST) to illuminate input sources of FIOs into bathing waters and consider un-culturable organisms is much needed. This project is based on samples collected during the 2022 summer bathing season; thus only trial data are available.

DNA will be extracted from samples already collected for existing monitoring methods, followed by both 16S rRNA gene based meta-barcoding and metagenomics sequencing using the minION Mk1c. Filters containing cultured organisms used for current FIO monitoring may also be extracted for Whole Genome Sequencing.

We will use metagenomes generated from a subset of these samples (sequenced using both Illumina and Oxford Nanopore platforms) to resolve metagenome-assembled-genomes (MAGs) from hybrid assemblies. Then, individual genome variants of species of interest can be identified, offering an extremely robust approach to MST. Generation of MAGs would illuminate both input sources of FIOs and help define links with FIOs and their capacity for causing human illness, as well as provide useful predictions on decay rate and cell viability in different conditions based on ecology.

These data inform stakeholders of the truest monitoring of bathing waters, meaning decisions can be made based on what is contributing most to FIOs and most importantly, safety of bathing waters can be accurately reported over the summer seasons.

P15

Bacterial whole genome sequencing to determine genetic basis of adaptation to the mosquito gut.

Laura Brettell[1], Vishaal Dhokiya[1], Holly Nichols[2], Aidan Foo[1], Miguel Medina Munoz[2], Kerri Coon[2], Grant Hughes[1], Eva Heinz[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [2]University of Wisconsin-Madison, Madison, USA

Abstract

The mosquito gut microbiome is vital for host survival and has profound effects on host fitness and vectorial capacity. Determining genomic features that allow bacterial to be successful colonizers of the mosquito gut provides novel insights into host adaptation and is vital for the development of paratransgenesis approaches to combat the spread of mosquito-borne pathogens. Here, we carried out whole genome sequencing on >400 bacteria samples isolated from *Aedes*, *Anopheles* and *Culex* mosquitoes. We then took forward genomes of key genera commonly associated with the mosquito gut including *Serratia, Acinetobacter, Enterobacter* and *Cedacea*. We identified genomic differences between mosquito-associated and environmentally-derived isolates. This collection of bacterial isolates with associated genomes will now be expanded to include contributions from across the world. The resource will be open to use for the scientific community to use in experimental approaches to answer diverse questions on microbial assembly and evolution in the mosquito gut and will facilitate wide-reaching collaborations.

P16

Staphylococcus haemolyticus is a reservoir of antibiotic resistance genes in the preterm infant gut microbiome

Lisa Lamberte[1], Raymond Kiu[2], Robert Moran[1], Heather Felgate[2], Mark Webber[2], Lindsay Hall[2,3,4], Willem van Schaik[1]

[1]Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom. [2]Quadram Institute Bioscience, Norwich, United Kingdom. [3]School of Life Sciences, Technical University of Munich, Freising, Germany. [4]ZIEL - Institute of Food and Health, Technical University of Munich, Freising, Germany

Abstract

Staphylococcus haemolyticus is commonly found on human skin. It is important in preterm infants as one of the leading causes of late-onset sepsis. There are significant gaps in our knowledge of the diversity of *S. haemolyticus* strains that colonise preterm infants.

To address this, we isolated *S. haemolyticus* from stool samples of a cohort of preterm infants in the multi-centre BAMBI (Baby Associated MicroBiota of the Intestine) study. We then used a combination of genomics and experimental approaches to determine the diversity of *S. haemolyticus* in this cohort.

Phylogenetic analysis using short-read whole genome sequences of *S. haemolyticus* isolates from our preterm infant cohort (compared with publicly available *S. haemolyticus* genomes) suggested that *S. haemolyticus* can be divided into three distinct phylogenetic clades, with one of these consisting mostly of isolates from the preterm infant cohort. The gene *aacA-aphD* that confers gentamicin resistance was found to be present in 53/57 (93%) of isolates from the preterm infant gut. Hybrid assemblies using long-read sequencing data generated complete genomes for representative isolates of the three major clades. These showed that *aacA-aphD* are in the transposon, Tn*4001*, which was found in a variety of genetic contexts, suggesting that it has been acquired independently on multiple occasions. Hybrid assemblies also show two different plasmid configurations that are unique compared to previously characterised *S. haemolyticus* sequences.

Our data show that *S. haemolyticus* isolates from the preterm infant gut carry multiple antibiotic resistance genes and may form a unique sub-population within the species.

P17

Metagenomics binning by coverage and composition for long reads

Anuradha Wickramarachchi[1], Vijini Mallawaarachchi[2], Vaibhav Rajan[3], Yu Lin[4]

[1]Commonwealth Scientific and Industrial Research Organisation, Westmead, Australia. [2]Flinders University, Bedford Park, Australia. [3]National University of Singapore, Singapore, Singapore. [4]Australian National University, Canberra, Australia

Abstract

Metagenomics studies have provided key insights into the composition and structure of microbial communities found in different environments. Among the techniques used to analyse metagenomic data, binning is considered a crucial step to characterise the different species of micro-organisms present. The use of short-read data in most binning tools poses several limitations, such as insufficient species-specific signal, and the emergence of long-read sequencing technologies offers us opportunities to surmount them. However, most current metagenomic binning tools have been developed for short reads. The few tools that can process long reads either do not scale with increasing input size or require a database with reference genomes that are often unknown.

We present MetaBCC-LR, a scalable taxonomy-independent binning tool to bin metagenomic long reads. MetaBCC-LR uses discriminatory features, that capture species abundance and composition information, to determine the number of bins and develop a statistical model for each bin. We evaluate MetaBCC-LR on multiple simulated and real metagenomic long-read datasets with varying coverages and error rates. Our experiments demonstrate that MetaBCC-LR substantially outperforms state-of-the-art reference-free binning tools, achieving ∼13% improvement in F1-score and ∼30% improvement in ARI compared to the best previous tools. Moreover, we show that using MetaBCC-LR before long-read assembly helps to enhance the assembly quality while significantly reducing the assembly cost in terms of time and memory usage. The efficiency and accuracy of MetaBCC-LR pave the way for more effective long-read-based metagenomics analyses to support a wide range of applications.

MetaBCC-LR is freely available on GitHub at https://github.com/anuradhawick/MetaBCC-LR.

P18

Mechanisms of genetic variations in Bacteroides phages

Bhavya Nalagampalli Papudeshi[1], Vijini Mallawaarachchi[1], Alejandro Vega[2], Jody McKerral[1], Michael Roach[1], Sarah Giles[1], Clarice Cram[1], Evan Pargin[1], Will Plewa[1], Cole Souza[2], Katelyn McNair[2], Maria Fernanda Mora[2], Anca Segall[2], Elizabeth Dinsdale[1], Robert Edwards[1]

[1]Flinders University, Adelaide, Australia. [2]San Diego State University, San Diego, USA

Abstract

Bacteroides are the most common bacteria in the human gut, and they degrade many complex polysaccharides that would otherwise remain undigested. The abundance of Bacteroides in the gut is shaped by the phages that infect and kill them. In response, bacteria alter their cell wall composition to limit phage infectivity. We have isolated and sequenced three Bacteroides isolates that were infected by 30 phages. The bacterial genomes reflect the availability of sugars in the human gut. Bacteriophages demonstrate different approaches to overcome bacterial defences. We demonstrate selective codon reassignment in novel isolates of beta-crAssphage that infects Bacteroides cellulosilyticus, hypermutability, and novel DNA modifications in phages that infect Bacteroides fragilis and Bacteroides uniformis. We also discovered a new highly abundant Bacteroides phage unrelated to crAssphage and demonstrate its global diversity. The arms race between phage and bacteria that has raged for millions of years has selects for rapid evolution of host and prey, and these genomes reveal this unending conflict.

P19

Using assembly graphs to identify bacteriophage genomes in metagenomic samples

Vijini Mallawaarachchi, Michael Roach, Bhavya Papudeshi, Elizabeth Dinsdale, Robert Edwards

Flinders University, Adelaide, Australia

Abstract

Microbial communities found within the human gut have a strong influence on human health. Gastrointestinal diseases such as inflammatory bowel disease (IBD) are driven by intestinal bacteria and viruses. Viruses infecting bacteria, known as bacteriophages, play a key role in modulating bacterial communities residing within the human gut. However, the identification and characterization of novel bacteriophages in microbiomes remains a challenge.

High-throughput metagenomic sequencing has enabled the study of uncultivated microbial and viral communities. There are many tools to identify viral sequences from metagenomic data. These tools often make use of similarity between sequences, nucleotide composition, and the presence of viral genes/proteins. Most existing tools consider the individual sequences and determine whether they are of viral origin. Due to the challenging nature of viral assembly, their genomes can be fragmented, and per-sequence based viral identification tools may not produce optimal results.

Metagenomic assemblers build a structure known as the assembly graph by overlapping reads to produce longer sequences called contigs. Genomes typically correspond to long paths within the assembly graph, and contigs of connected components are more likely to belong to the same genome. Hence, the assembly graph retains connectivity and neighbourhood information within fragmented assemblies. Previous studies have made use of assembly graphs in metagenomic binning, mostly to identify bacterial genomes. This work introduces how assembly graphs can be used to identify viral genomes from fragmented metagenomic assemblies. Specifically, we demonstrate the use of assembly graphs to resolve bacteriophage genomes within metagenomic samples collected from patients with IBD.

P20

Muricauda antarctica genome assembled from Epidermal microbiomes of leopard sharks (*Triakis semifasciata*) across captive and wild environments

Asha Goodman[1], Bhavya Papudeshi[2], Michael Doane[3], Elizabeth Dinsdale[3]

[1]San Diego State University, San Diego, USA. [2]Flinders Institute, South Adelaide, Australia. [3]Flinders University, South Adelaide, Australia

Abstract

Characterizations of sharks-microbe systems in wild environments have outlined patterns of species-specific microbiomes. However, whether captivity affects these trends has yet to be determined. We used high-throughput shotgun sequencing to assess the epidermal microbiome belonging to leopard sharks (*Triakis semifasciata*) in captive (Birch Aquarium, La Jolla California born and held permanently in captivity, n = 4), semi-captive (held in captivity for <1 year in duration and scheduled for release; Scripps Institute of Oceanography, California, n = 4) and wild environments (n = 19). We report captive environments do not drive epidermal microbiome compositions of *T. semifasciata* to significantly diverge from wild counterparts (PERMANOVA Genus level, Pseudo-F df =2, 27 =, P(perm) > 0.1) as life-long captive sharks maintain a species-specific epidermal microbiome resembling those associated with semi-captive and wild populations. Cross assembly of the 27 *T. semifasciata* metagenomes yielded 54 metagenome assembled genomes (MAGs) containing 241 814 contigs greater than 1 kilobase pairs, with N50 of 735 bp and N75 of 583 bp, and two (Bin 27 and Bin 9) were identified as belonging to the Muricauda genus, an increasingly classified child taxon of Flavobacteriaceae family. The contribution of annotated MAGs from each group ranged from 5.5% ± 1.8 S.D. from captive hosts to 87% ± 0.66 from wild individuals, with semi-captive contributing 7.5% ± 1.1 to MAG generation. Our report illustrates that epidermally-associated microbes belonging to *T. semifasciata* have similar diversity, marginal, changes in specific functional genes and retain microbes across environments.

P21

Microbiome drift predicts metabolic differences in replicate experiments

Abbie Williams[1], Katie Bull[1], Nicola Beesley[2,3], Catherine Hartley[2], Diana Williams[2], Jane Hodgkinson[2], Mick Bailey[1], Laura Peachey[1]

[1]Bristol Veterinary School, University of Bristol, Bristol, United Kingdom. [2]Department of Infection Biology and Microbiomes, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [3]Department of Biological Sciences, University of Chester, Chester, United Kingdom

Abstract

Studies have demonstrated the importance of the gastrointestinal (GI) microbiota in regulating mammalian immune responses and metabolism. Helminth parasites exert immunomodulatory control over the GI microbiome, but such studies are often confounded between laboratories or replicates. The parasitic trematode, *Fasciola hepatica* (Liver fluke) is a huge burden on the livestock industry, and infection of sheep causes substantial production losses. We studied the effects of *F. hepatica* infection on ovine GI microbiomes using an initial large group of lambs randomised into four replicate pens containing both infected and uninfected lambs. Faecal samples were collected across a 13-week period and analysed. The strength of this design was its ability to concurrently measure pen effects and infection effects within the microbiome.

Remarkably, we found that faecal microbiomes were more determined by pen than by infection and diverged throughout the experiment. Predicting population metagenomes with PICRUSt2 demonstrated that divergence in faecal microbiomes was linked to likely changes in metabolic potential, rather than like-for-like substitution. Divergence of microbiomes at the pen level were reflected in alpha diversity, beta diversity, and differential abundance analysis, with effects becoming more pronounced across time.

Our results suggest that microbiomes in replicate experiments may diverge, particularly in longitudinal studies. This divergence may arise from simple stochastic drift from a common microbiome in isolated populations over time or could arise from “seeded” differences: the impossibility of truly randomising all components of intestinal microbiomes at the beginning of the study, resulting in small baseline differences that can become more pronounced over time.

P22

Microbiome stability and specificity of two marine sponge species

Claire Lamb, Joy Watts

University of Portsmouth, Portsmouth, United Kingdom

Abstract

Marine sponges are dominant and functionally important members of marine benthic systems, well known to harbour complex, abundant and diverse symbiotic microorganisms as part of their often species-specific microbiome. Microorganisms are good indicators for stress in marine environments as they are sensitive to minor environmental shifts due to their physiological thresholds and thus respond quickly to environmental change. Even minor variations in environmental conditions can have significant effects on organisms and their physiology. Changes in the sponge microbiome have previously been observed in relation to environmental changes including nutrient availability, temperature and light. Next generation sequencing of two marine sponges native to the U.K. (Hymeniacidon perlevis, Suberties massa) was performed at two different temperatures. A stable, host-specific microbiome was observed in each sponge species. Diversity within S. massa was dominated by one family, Terasakiellaceae, with the remaining communities also being detected in the associated seawater. H. perlevis demonstrated several sponge enriched bacterial families including Terasakiellaceae, Rhodobacteraceae and Sphingomonadaceae with the overall diversity differing distinctly from the community composition of the surrounding water column. The presence of a core sponge microbiome identified in each sponge species was not disrupted by temperature changes. Further studies are underway to examine specific metabolic pathways active in these symbiotic communities.

14–16 Meredith Street,
London, EC1R 0AB, UK
microbiologysociety.org

The Microbiology Society is a membership charity for scientists interested in microbes, their effects and their practical uses. It is one of the largest microbiology societies in Europe with a worldwide membership based in universities, industry, hospitals, research institutes and schools.

Our members have a unique depth and breadth of knowledge about the discipline. The Society's role is to help unlock and harness the potential of that knowledge.