Seeing Beyond Culture, Next generation sequencing for Food Safety and Environmental Monitoring

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Introduction
Optimizing Metagenomics at every step of the workflow

Founded by Professor Rita Colwell, CosmosID leverages over 13 years of R&D and experience to deploy optimized metagenomics workflows for different sample types in a wide range of study applications by addressing common bottlenecks in metagenomics.
End-to-End Metagenomics Solutions

To address uncertainty in lab-based metagenomics workflows as well as inefficiencies in modern-day bioinformatics, CosmosID supports industry, academia & clinicians with standardized and validated metagenomics solutions based on sample type and study intent, so that you can unlock the microbiome with confidence, on the first try!
What makes CosmosID unique?

- Metagenomics Specialist & Thought Leaders of 13+ years
- Optimized, Low-Bias Workflows by Sample Type
- Quality Management System & CLIA/GCP Compliance
- Industry-Leading Bioinformatics
- User-Friendly Software for Microbiome Data
- Extensive Experience in Clinical Trials – Kits, Logistics & Analysis
- Global Presence – Clients in 30+ Countries and Labs in 2 Continents
CosmosID & Food Safety
Sprouts have been implicated in foodborne illness outbreaks

- Can next-generation sequencing be used to detect potential sources of foodborne illness?

Goal: to examine the microbiome in sprout irrigation water in order to detect possible pathogens and antimicrobial resistance genes

Methods:
- DNA was extracted from baseline irrigation water and spent irrigation water
- Whole genome Illumina sequencing was performed
- The CosmosID pipeline was used to annotate bacteria, fungi, and antimicrobial resistance genes; a custom pipeline was used to annotate protists
Water Microbiota from a Commercial Alfalfa Sprout Crop over 4 Days of Growth

• Bacterial and fungal diversity increased

• Protist and AMR diversity remained fairly constant

• Dominant bacteria include: *Klebsiella*, *Pseudomonas*, *Acinetobacter*, and *Enterobacter*

• Genes for aminoglycoside and β-lactam resistance declined in abundance over the 4-day sampling period, and other types of resistance observed at earlier time points (such as resistance to vancomycin, trimethoprim, and macrolide resistance) disappeared by day 4

NGS can potentially contribute to understanding how contamination by human pathogens and resistance genes occurs
Temporal Resistome and Microbial Community Dynamics in an Intensive Aquaculture Facility with Prophylactic Antimicrobial Treatment

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• Aquaculture is routinely prophylactically treated with antibiotics, which may contribute to the spread of antimicrobial resistance

• Goal: identify seasonal patterns of antimicrobial resistance and bacterial community composition in water of a prophylactically-treated silver carp aquaculture pond

• Methods: Eight water sample profiles were taken from the aquaculture ponds in triplicate; DNA was extracted and whole genome shotgun sequencing performed; the CosmosID pipeline was used to identify bacterial, virus, protist, fungi and antimicrobial resistance gene community composition
Temporal Resistome and Microbial Community Dynamics in an Intensive Aquaculture Facility with Prophylactic Antimicrobial Treatment

- Increased relative abundance of sulfonamide and tetracycline resistance genes in fishpond-03
  - No differences observed for genes encoding resistance to antimicrobials that were not used in the fishpond-03.

- Seasons strongly dictated bacterial community composition, with high abundance of cyanobacteria in summer and increased relative abundance of Flavobacterium in the winter

- Prophylactic use of sulfonamides in aquaculture should be restricted

Seasonal monitoring can potentially help determine the best ways to reduce use of antimicrobials, while protecting the aquaculture
A New Whole Genome Culture-Independent Diagnostic Test (WG-CIDT) for Rapid Detection of *Salmonella* in Lettuce

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- Rapid detection of foodborne illness could revolutionize food safety and help prevent outbreaks
- Goal: development of a sensitive, whole genome shotgun sequencing-based rapid method for detection of as little as 1 colony forming unit of *Salmonella enterica* serovar Typhimurium spiked on 25g of lettuce
- Methods: Lab-grown *Salmonella enterica* serovar Typhimurium was diluted to $10^8$ cfu/ml and spiked onto lettuce; DNA was extracted and whole genome sequencing performed on the Ion Torrent platform; kmer based taxonomic identification was performed using the CosmosID pipeline

https://doi.org/10.3389/fmicb.2020.00602
A New Whole Genome Culture-Independent Diagnostic Test (WG-CIDT) for Rapid Detection of *Salmonella* in Lettuce

- Test successfully detects *Salmonella enterica* contamination in lettuce
  - All spike samples detected \geq 1 cfu
  - \geq 6M reads per sample
  - Non-spiked samples did not show *Salmonella enterica*
  - Test is rapid and sensitive

Has the potential to be universally applicable for any microbial contaminant on lettuce

**FIGURE 6** | Metagenomic detection of contaminants on the surface of lettuce. Heat map shows the detection of *Salmonella* in spiked sample (*) but not in a non-spiked control.
Genotypic and phenotypic characterization of multidrug resistant Salmonella Typhimurium and Salmonella Kentucky strains recovered from chicken carcasses

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• Salmonella Typhimurium is the leading cause of gastroenteritis in the USA, and the Kentucky strain is most commonly recovered from commercially processed poultry carcasses

• Goal: compare the genotypic and phenotypic properties of two Salmonella strains recovered from commercially processed chicken carcasses using whole genome sequencing, phenotype characterizations and a killing assay

• Methods: DNA was extracted from S. Typhimurium and S. Kentucky and whole genome sequenced on the Illumina MiSeq; a phylogenetic tree was created along with 28 other S. enterica strains to establish evolutionary relationships; SNP analysis was performed; two methods were used for whole genome comparison to identify similarities and differences between the strains

https://doi.org/10.1371/journal.pone.0176938.g001
Genotypic and phenotypic characterization of multidrug resistant Salmonella Typhimurium and Salmonella Kentucky strains recovered from chicken carcasses

- ST221_31B and SK222_32B genomes into distinct monophyletic serovar clades
- The genomes of ST221_31B and SK222_32B harbor several genomic regions with significant genetic differences including:
  - Phage and phage-like elements
  - Carbon utilization or transport operons
  - Fimbriae operons
  - Putative membrane associated protein-encoding genes
  - Antibiotic resistance genes
  - Siderophore operons
  - Numerous hypothetical protein-encoding genes

*Salmonella* Typhimurium and *Salmonella* Kentucky are distinct strains; Typhimurium may be better able to survive host defenses and be more invasive.
Data Analysis
(is not the bottleneck)
Automated Bioinformatics with Strain-Level Resolution

World’s Largest Curated Database: 170,000+ Microbial Genomes & Gene Sequences

Proprietary Algorithm: Industry-Leading Sensitivity, Specificity & Precision

Machine Learning Filters: Lowest False-Positive Rates

Multi-Kingdom ID: Bacteria, Viruses, Phages, Fungi, Protists, AMR, VF, Functions

70+ Publications

Winner Of 3 Community Benchmark Challenges (2x FDA and Janssen Mosaic)

In-House Benchmarking: Sub-species Resolution

• Genome Biology Publication
• Author-Approved CosmosID Blog
• www.cosmosid.com/literature
User-Friendly Data Access via CosmosID-HUB

Software developers at CosmosID have automated these award-winning pipelines and made them available through a user-friendly and interactive web-based application which means you can interpret your data straight away within the app and/or set up command line workflows for convenient movement of data!

Create a free account at app.cosmosid.com!
Broiler Chicken Gut Microbiome Analysis

Original Study (Hou 2016)

• Used 16S rRNA and whole genome sequencing to compare divergently selected lean line and fat line broiler chickens for bacterial and functional differences
  • Fourteen differentially abundant genera were identified, including known short chain fatty acid producers, which were depleted in the fat line chickens
  • More taxa were enriched in fat line chickens, including the potentially pathogenic Enterococcus
  • Functions for amino acid transport and metabolism, nucleotide transport and metabolism, coenzyme transport and metabolism, and lipid transport and metabolism were overrepresented in the lean line chickens

• Conclusions: while some significant structural differences were observed among taxonomic and functional composition, the study cannot determine whether these differences contribute to obesity in the chickens
Taxonomic and Antimicrobial Resistance Results
No Significant Differences in Diversity Among Lean vs Fat Chickens

- No significant differences by alpha or beta diversity
- Lean and fat chicken groups represent similar bacterial diversity and are not dissimilar from each other in bacterial community composition
Gut Microbiome Comparison of Fat vs Lean Broiler Chickens

- 28 chicken gut microbiome samples compared
  - 14 lean
  - 14 fat
- No clear clustering by body mass
Fat Chickens are Enriched with *Enterococcus* and *Staphylococcus aureus*

- Applied linear discriminant analysis effect size (LEfSe) to identify differentially abundant taxa between the groups
- Three taxa are enriched in lean chickens and 10 in fat, including *Enterococcus* and *Staphylococcus aureus*
Fat Chickens are Enriched in Antimicrobial Resistance Genes

- 3 genes are differentially abundant in lean chickens
- 9 genes are differentially abundant in fat chickens
- Fat chickens are more likely to harbor bacteria resistant to phenicols, sulphonamides, and aminoglycoisdes
- Both cohorts have AMR genes for tetracylines, macrolides and beta-lactamases
Functional Results
No Significant Difference by Alpha or Beta Diversity

• Similar to the taxonomic results, there are no significant differences by alpha or beta diversity among functions
  • Fat line chickens have slightly higher alpha diversity, although this is not significant
Some Patterns in Functions Can Be Observed

- Here, the chickens do not cluster exclusively by cohort, implying that there are not distinct differences by function
- Some differences in less abundant functions can be observed between individual chickens
Fat Chickens are Enriched in Fatty Acid and Amino Acid Synthesis Pathways

- Lean chickens are enriched in fewer functions
  - These include amino acid and glycogen biosynthesis pathways
- Fat chickens are enriched in several fatty acid biosynthesis pathways
  - These may indicate increased fat storage activity in fat chickens
Conclusions

• No broad differences in alpha or beta diversity were observed between fat and lean chickens but specific differences were identified
  • Specific taxa and AMR genes were enriched in each group
  • The potential pathogens *Enterococcus* and *Staphylococcus aureus* were enriched in fat line chickens
  • Fat line chickens were enriched for antimicrobial resistance genes
  • Fat line chickens were enriched in fatty acid biosynthesis pathways

• This suggests that there may be subtle differences between the lean and fat groups at the taxonomic and functional levels rather than broad overall patterns
  • Fat chickens may harbor more pathogens and antimicrobial resistance genes; it is unclear if this is associated with selection for obesity or prophylactic treatment with antibiotics (either directly or at low levels in feed)
  • Can we use supplements instead of antimicrobials to prevent AMR resistance transfer to humans?

• Analysis and figures were generated in less than two hours on the CosmosID-Hub
Additional Use Cases

• Virtual panels for targeted pathogen detection from shotgun sequencing

• Spoilage risk and prediction

• Factory source tracking and monitoring

• Field based Diagnostics
It’s time to use Next Generation Sequencing for Food Safety and Environmental Monitoring at Scale!

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