Techniques in Paleomicrobiology for the study of *Mycobacterium leprae*

Kieran Milner, MSc. Student
Supervisors: Meenu Sharma, Denice Bay
November 2018
Presentation Outline

1) Overview of Mycobacteria

2) Leprosy Pathogenesis

3) Paleomicrobiology

4) Case Studies
Mycobacteria
Mycobacteria

- **Intracellular Pathogens** – eg. *Mycobacterium tuberculosis* and *Mycobacterium leprae*

- **Acid-Fast Bacilli** – retain carbol fushin stain after acid alcohol wash

- **Unique Cell Envelope** – contains mycolic acids
Types of Bacterial Cell Envelopes

Gram-Negative
- Outer Membrane
- Thin Peptidoglycan
- Inner Membrane

Gram-Positive
- Thick Peptidoglycan
- Single Membrane

Mycobacteria
- "Outer Membrane"
- Arabinogalactan
- Peptidoglycan
- Inner Membrane

Referenced from: Brown et al. 2015.
Mycobacterial Pathogens

1) **Tuberculosis** – *Mycobacterium tuberculosis*
   - Remains the number one cause of death from an infectious disease today

2) **Leprosy** – *Mycobacterium leprae*
   - Cases have declined since the introduction of multi-drug therapy in 1981
   - Yet, burden is still high in developing countries throughout Africa, Asia, and South America
   - 200,000 new cases annually
Mycobacteria are Slow-Growing Organisms

Generation Times:

• *Escherichia coli* – 20 minutes

• *Mycobacterium tuberculosis* – 12-24 hours

• *Mycobacterium leprae* - 12-14 **days**
Mycobacterium leprae
Leprosy

• Infects humans, primates, armadillos, and rodents

• **Early Stages**: lesions, rashes, nerve damage

• **Late Stages**: permanent deformities including physical changes in bones
M. leprae Infects Multiple Cell types

Schwann Cell

Dermal Macrophage
Leprosy

- Multi-drug therapy has historically been effective

- The emergence of antimicrobial resistance has made leprosy harder to treat

- Longer and less effective treatment outcomes

Referenced from: Suzuki K et al. 2012.
Multi-Drug Therapy for Leprosy

- Rifampin
- Dapsone
- Clofazimine
Paleomicrobiology
Paleomicrobiology

- Analysis of biomarkers in archaeological samples (eg. DNA, lipids)
- Useful for the study of *M. leprae* – cannot be grown in vitro
- Main Issues: contamination, DNA degradation
Late Stages of Leprosy

- Osteoporosis, bone remodeling: both destruction and thickening

- Most prominent in limb and facial bones – closely associated with the peripheral nervous system allowing *M. leprae* invasion

Referenced from: Taylor GM et al. 2013
Contamination

1) During Sample Collection
   • Gloves, fully body gowns
   • Strict methods, metadata

2) During Lab Analysis
   • Proper sterilization
   • Suicide PCR
   • Independent replication

Ancient DNA Damage

1) Fragmentation – 100 bp fragments

- *M. leprae* DNA is protected by the impermeable mycobacterial cell envelope – results in longer fragments (eg. 500 bp)
Ancient DNA Damage

2) Cytosine Deamination – causes misincorporations

- Treatment with uracil-DNA-glycosylase and endonuclease VII repairs damaged DNA and improves accuracy during amplification
Paleomicrobiology Methods

• 1) Sample Collection

• 2) Screening, Enrichment
  • Eg. PCR, Hybridization Capture

• 3) DNA Sequencing – Illumina

• 4) Bioinformatics
  • Alignment, reference mapping, SNP/InDel calling
  • Maximum parsimony trees, Bayesian trees
Lipid Biomarkers

Fluorescence High-Performance Liquid Chromatography

- Mycolic acids are much more stable than DNA
- Species-specific lipid profiles distinguish leprosy from other mycobacteria
Case Studies
Case Study 1 – Schuenemann et al. 2018

Ancient Genomes Reveal a High Diversity of *Mycobacterium leprae* in Medieval Europe

- Analysis of published + 10 new genomes of *M. leprae* from medieval Northwestern Europe

- DNA extraction -> Screening -> (Enrichment) -> Amplification -> Sequencing -> Bioinformatics

- Evidence of transmission from England Red Squirrels, and multiple introductions of *M. leprae* into Europe

Case Study 2 – Benjak et al. 2018

Phylogenomics and Antimicrobial Resistance of the Leprosy Bacillus *Mycobacterium leprae*

- Extraction of *M. leprae* DNA from patient skin biopsy samples

- DNA extraction -> Screening -> (Enrichment) -> Amplification -> Sequencing -> Bioinformatics

- SNP data can be used to predict antimicrobial resistance phenotypes, Diversity of *M. leprae* genomes predicts human migration patterns

Referenced from: Benjak A et al. 2018.
Concluding Remarks

• Paleomicrobiology is useful for the study of ancient diseases

• Allows researchers to track the spread and origin of leprosy

• Furthers our understanding of disease dissemination, evolution, and antimicrobial resistance
References

Questions