Characterising the (epi+) Genome of *Legionella longbeachae* Serogroup 1 Clinical Isolates

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Legionnaires’ Disease

- Pneumonia caused by *Legionella* bacteria
- Can be severe and life threatening
- No human-to-human transmission
- Requires specific antibiotic treatment
- Notifiable disease
- *L. longbeachae* predominant cause in NZ-soil/composted plant material
Potting mix nearly kills Wellington man

RHANNON MCCONNELL
Last updated 18:52, February 17, 2016

Legionnaires' puts 10 Aucklanders in hospital

AMY MAAS

Legionnaires' disease puts him in intensive care

Tom Kitson - 19.08, Oct 05 2018

Health boards, including Hutt Valley DHB, seeing more cases of legionellosis

BLAKE CRAYTON-BROWN
Last updated 08:14, January 23 2016

How to protect yourself against Legionnaires' disease

CATHY STEPHENS
Last updated 11:37, November 21 2017

Spring garden chores linked to spike in Legionnaires' disease

KATIE NEWMAN
Last updated 17:43, October 9 2018

Half of Legionnaires' disease sufferers may be going undiagnosed

Madison Northcott • 15:39, Nov 17 2017

Gardening can make you sick
**L. Longbeachae genome**

- Until 2017 isolate NSW 150 only complete genome sequence
  - 4.1 Mb chromosome
  - 71 kb plasmid

**Objective**

- Assess the geo-temporal genetic diversity of *L. longbeachae* clinical isolates from around NZ over a 22 year period (1993-2015)
Sequencing and NZ Ref Genome

- Isolates sequenced on Illumina MiSeq (short-read-250 bp PE)
- One isolate (F1157CHC-2014, Canterbury Isolate) selected for further sequencing to get NZ Ref genome-used PacBio RSII Platform (long-read, 15,000 kb library cut-off)
- PacBio also provides epigenome data
- Isolate had chromosome of ≈ 4.1 Mb and a plasmid of ≈108 kb
- 3,577 coding sequences in chromosome and 114 in plasmid
Chromosome Architecture

Genes on plus strand
Genes on minus strand

Snippy defined recombination areas
SNPs in recombination areas

SNPs are in recombinant areas
Non-strand
Non-strand SNPs

m4C consistent with gene strand
m4C inconsistent with gene strand

All m4C

m6A consistent with gene strand
m6A inconsistent with gene strand

All m6A

GC % Snippy defined recombination areas
Repeats - forward
Repeats - palindromic
Repeats forward
Recombination areas
Plasmid
Geo-temporal analyses

- The 54 sg1 isolates analysed shared 5,383 core SNPs. Only 2,338 were non-recombinant.

- Phylogenetic modelling of non-recombinant SNPs estimated that isolates mutated at a rate of $3.45 \times 10^{-8} - 1.71 \times 10^{-7}$ substitutions/site/year.

- Isolates had a shared common ancestor between 1034 BCE and 1619 CE.

- One large clade and one small clade were observed. The largest clade consisted of isolates from multiple regions, while the second clade contained isolates only from Canterbury.
Epigenetic Modification

- Epigenetic = “on top of”
- Methylation 6mA, 4mC and 5mC
- Restriction modification system-protects against viral DNA insertion
Summary

• Most variability in the *L. longbeachae* genome is from recombination and there have been large-scale rearrangements.

• Two highly related clades occur throughout NZ and persist over time.

• Most genetically distinct clade consisted of isolates only from Canterbury BUT oversampling from this region.

• Mosaic plasmids-intra and inter-species exchange

• Epigenic modification- highly modified genome, m6A most common, m4C modified bases conserved
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