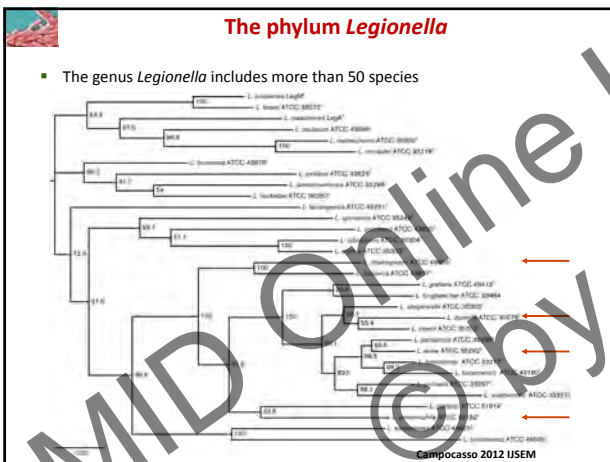
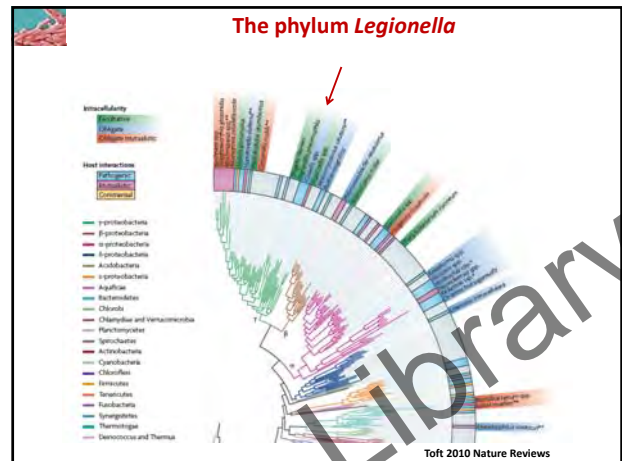


Legionella genomics

Claire Bertelli

Intracellular Bacteria: From Biology to Clinic
Villars-sur-Ollon, August 2013

Unil
UNIL Université de Lausanne
SIB

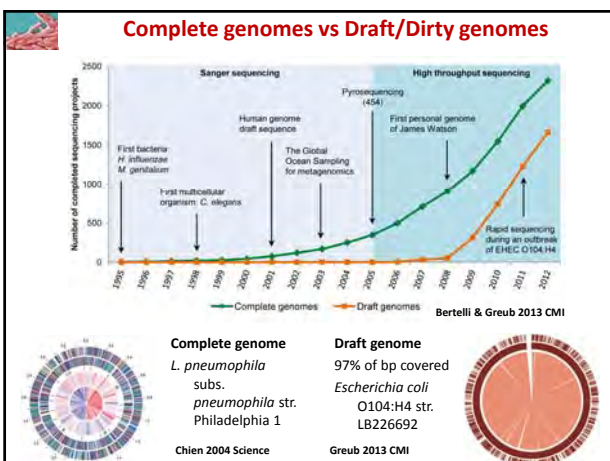


Legionella genomes

The genome sequences of 6 *Legionella* species are available

	Complete genomes	Draft – Dirty genomes	No data available yet
<i>Legionella pneumophila</i>	12 strains	1	13
<i>Legionella longbeachae</i>	1	1	
<i>Legionella anisa</i>		1	1
<i>Legionella drancourtii</i>		1	
<i>Legionella shakespearei</i>		1	
<i>Legionella</i> sp.		1	1

Registered projects for *Legionella drozanskii*, *Legionella fallonii*, *Legionella hackeliae*, *Legionella moravica*, *Legionella rowbothamii*



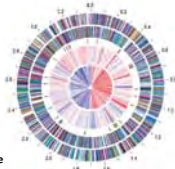
Genomic features

	<i>Legionella pneumophila</i>	<i>Legionella longbeachae</i>	* <i>Legionella anisa</i>	* <i>Legionella drancourtii</i>	* <i>Legionella shakespearei</i>	* <i>Legionella</i> sp.
Genome size (Mbp)	3.4	4.15	4.32	4.15	3.51	3.37
%GC	38.4	37.1	38.2	38.6	41.7	39.9
No of rRNA operons	3	4	n.a.	n.a.	n.a.	n.a.
No of proteins	3166	3470	3847	3959	3000	3394
Plasmid	+ / -	+	n.a.	n.a.	n.a.	n.a.

* : draft genome, n.a.: information not available

The first genome: *Legionella pneumophila* strain Paris

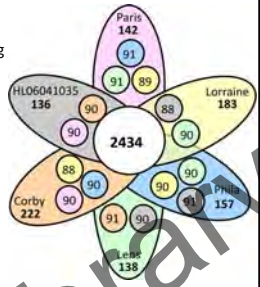
- More than 350 binding proteins and permeases, to utilize nutrients within diverse environments
- Homologs to eukaryotic genes
- Plasmid like element of 45kb that exists as an episome or within the chromosomes
- Phage-derived genes and insertion sequences (mobile elements): 2.4% of the genome
- lcm/dot* genes encoding for the type IVB secretion system
- Lvh/lvr* cluster encoding for the classical type IV secretion system
- No particular expansion of gene families
- Traces of horizontal gene transfers



Chien 2004 Science

Legionella core genome and pan genome

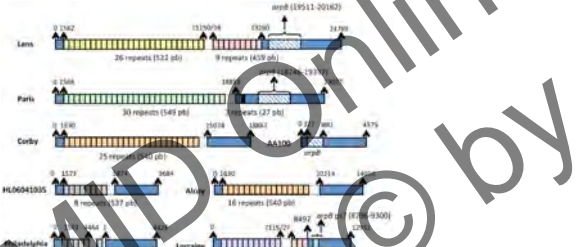
- Highly conserved and syntenic core genome that comprises many eukaryotic-like proteins (n=46) and proteins harboring eukaryotic motifs (n=39)
- Highly dynamic accessory genome of about 300 genes mainly formed by mobile genetic elements
- Conserved repertoire of over 200 Dot/Icm type IV secreted substrates.



Gomez-Valero 2011 BMC genomics

Occurrence of recombination

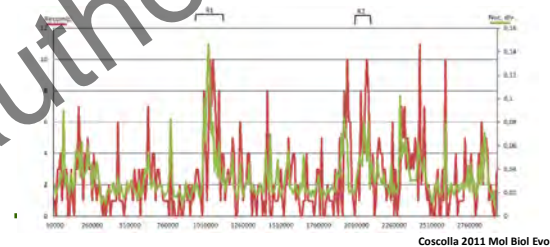
- Analyses of SNPs distribution suggests that large fragments (>200 kbs) are exchanged between *L. pneumophila* strains and contribute to the genome dynamics in the natural population.
- Example: *rtxA* gene that contains a long tandem repeated domain of variable copy number and sequence



Gomez-Valero 2011 BMC genomics

Occurrence of recombination

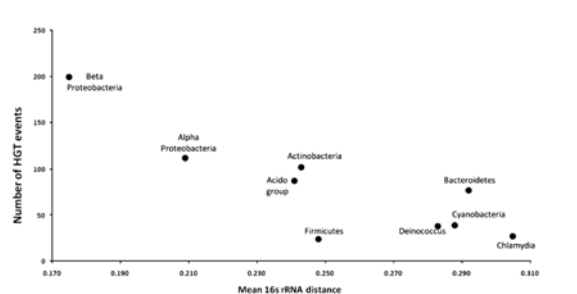
- Hot spot of gene recombination calculated from the comparison of 4 genomes of *L. pneumophila*



Coscolla 2011 Mol Biol Evol

Occurrence of horizontal gene transfer

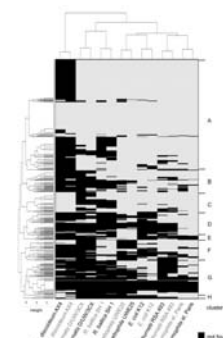
- Negative correlation between phylogenetic distance and likelihood of horizontal gene transfer.



Coscolla 2011 Mol Biol Evol

Horizontal gene transfer between amoebal pathogens

- Identification of orthologs in *L. drancourtii* and *Parachlamydia acanthamoebae*
- Presence/absence of orthologs in closely to more distantly related organisms
- Selection of genes absent from closely to distantly-related organisms
- Phylogenetic reconstruction with the 20 best BLAST hit of *L. drancourtii* and *P. acanthamoebae* proteins



Gimenez 2011 BMC Genomics

Horizontal gene transfer between amoebal pathogens

	No of horizontal transfers identified
<i>L. drancourtii</i> – <i>P. acanthamoebae</i>	7
<i>P. acanthamoebae</i> – Legionellales	18
Legionellales – Chlamydiales	4
<i>P. ac.</i> / <i>L. dr.</i> – Rickettsiales	9
<i>P. ac.</i> / <i>L. dr.</i> – Others	4

Gimenez 2011 BMC Genomics

Which mechanisms for horizontal gene transfer?

- Through secretion systems present by the occurrence of conjugal transfer

<http://www.mikrobiologie.uni-erlangen.de>

- Plasmids are exchanged among strains and circulate between different *Legionella* species

Genomic islands in *L. pneumophila* strain Corby

- Six genomic islands: LpcGI-1 and -2, LpcGI-Asn, LpcGI-Phe, Trb-1 and Trb-2

Lautner 2013 J Bact

Genomic islands in *L. pneumophila* strain Corby

- Genomic islands LpcGI-1, Trb-1 and LpcGI-Asn also exist as episomal forms but not LpcGI-Phe
- LpcGI-2 can form episomal rings A, B or AB
 - Episomal ring A formation depends on the integrase *lpc1833*
 - Mutants in *lpc1884* and *lpc2123* slightly increased the formation of ring B & AB

Lautner 2013 J Bact

Genomic islands in *L. pneumophila* strain Corby

- LpcGI-2 encodes a functional conjugation system and is horizontally transferred by a process mediated through *pilT* and *lpc1833*
- Trb-1 and Trb-2 have an *oriT* region and both can be excised from the chromosome forming episomal circles. Trb-1 episomal formation and conjugation depends on *int-1* gene
- Trb-1 can be horizontally transferred by conjugation and integrated into the genome in a site-specific manner within the *trnA(Pro)* gene.
- Mutants strains in *int-1* show no modification of intracellular multiplication in *A. castellanii*.

Glockner 2008 Gen Res
Lautner 2013 J Bact

Genomes, virulence and epidemics

Molecular base for the high prevalence of Sg1

- Differences in the O-antigen biosynthetic proteins
- LPS
 - Sg1 : classic ladder pattern; O-antigen is highly decorated.
 - Sg6 : minimally decorated and lack higher molecular weight species
- Variations in the outer-membrane O – antigen segment of the LPS can provide resistance to complement mediated serum killing in pathogenic bacteria

A Genomic maps of O-antigen biosynthetic genes for Sg1 and Sg6. Sg1 shows a classic ladder pattern with many genes, while Sg6 has fewer genes. Legend: Conserved genes (blue), Genes below 50% sequence homology (red), Unique Genes (grey).

B Agarose gel electrophoresis of LPS from Sg6 and Sg1. Sg1 shows a classic ladder pattern, while Sg6 shows a minimally decorated pattern.

C Bar chart showing the percentage of Sg1 and Sg6 surviving in serum at 24h and 120 min. Sg1 shows significantly higher survival than Sg6 in both conditions.

Khan 2013 PLoS One

Molecular base for the high prevalence of Sg1

- Serum Complement Proteins are able to bind to the surface of Sg6 but not Sg1
- Sg1 disseminates more efficiently than Sg6 in the A/J mice infection model

Bar chart showing Log₁₀ CFU of Sg1 (black bars) and Sg6 (white bars) in Lung, Blood, Liver, Spleen, and Kidney of A/J mice 48h post-intratracheal inoculation. Sg1 shows higher CFU counts in all organs compared to Sg6.

Khan 2013 PLoS One

Rapid genome sequencing in disease outbreak

- Pilot study to determine the feasibility of using bacterial WGS to differentiate outbreak from non-outbreak isolates collected during an outbreak of Legionnaires' disease.
- Limitations
 - small number of Legionella pneumophila isolates sequenced
 - limited number of published genomes for comparison
- Seven Legionella isolates
 - three clinical
 - four environmental
- Sequencing with Illumina MiSeq platform at Addenbrooke's Hospital, Cambridge.

Reuter 2013 BMJ Open

Rapid genome sequencing in disease outbreak

Sample number	Accession number	Biological origin	Type of sample	Serogroup	Monoclonal antibody subgroup	Sequence type*
Reference genome						
LP Philadelphia	AF017354.1	USA 1974	Clinical	1	Philadelphia	ST36
ATCC reference genome						
LP ATCC	CP007692.1	USA	Clinical	12	NA	ST187
LP Spain						
LP Spain	CP001828.1	Spain	Clinical	1	ND	ST578
LP Cook	CP000675.2	UK	Clinical	1	Knoville	ST51
LP Lens	CP028337.1	France	Clinical	1	Benidorm	ST15
LP Sigo	FR87201.1	USA	Clinical	1	Benidorm	ST42
LP Paris	CR62838.1	France	Clinical	1	Philadelphia	ST1
LP Lorraine	F0968210.1	France	Clinical	1	ND	ST47
LP H.06041925	F0958211.1	France	Environmental	1	ND	ST734
Outbreak investigator isolates						
LP03	ERS166051	Patient 1	Clinical	1	Philadelphia	ST37
LP06	ERS166045	Patient 2	Clinical	1	Philadelphia	ST37
LP17	ERS166047	Patient 3	Clinical	1	Altenborn/France	ST47
LP056	ERS166052	Site A cooling tower 1	Environmental	1	Philadelphia	ST37
LP427	ERS166050	Site A cooling tower 2	Environmental	1	Philadelphia	ST37
LP467	ERS166049	Domestic spa pool	Environmental	1	Philadelphia	ST37
LP423	ERS166048	Site B cooling tower 1	Environmental	1	Oxford/OLDA	ST1

*Sequence type was derived from the genome sequence data and was concordant with the results of the seven-allele sequence-based typing method. NA, Not applicable; ND, not determined.

Reuter 2013 BMJ Open

Rapid genome sequencing in disease outbreak

- Distinguish outbreak from non-outbreak isolates
- Confirm the probable environmental source
- In this outbreak, performance of WGS sequence was equivalent to conventional sequence-based typing in differentiating the outbreak from the non-outbreak strains
- Unable to distinguish the epidemiologically most likely source of the outbreak (site A cooling towers) from the domestic spa pool

Phylogenetic tree showing the relationship between outbreak (LP03, LP06, LP17, LP056, LP427, LP467) and non-outbreak (LP05, LP06, LP17, LP056, LP427, LP467) isolates. The outbreak isolates form a distinct cluster.

Reuter 2013 BMJ Open

Conclusion

- Comparative genomics, evolutionary and functional analyses suggested that
 - The interaction between Legionella and its amoebal host shaped this pathogen to conserve a broad host-range. In particular, host cycling might be a key step in maintaining pathways and preventing specialization.
 - Recombination and horizontal gene transfer are frequent in L. pneumophila
 - Horizontal gene transfer also occurs with more distantly-related prokaryotes also less frequently, including with other intracellular and intra-amoebal pathogens. These transfer may occur within the amoebal host.
- Genome sequencing is an essential tool to investigate the molecular bases for the success of some serogroups or some clones
- Rapid genome sequencing may be used to investigate current and past outbreaks



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