Villars-sur-Ollon, Switzerland

ESCMID Postgraduate Technical Workshop
Intracellular Bacteria: From Biology to Clinic

29 August 2013

Genomics of *Tropheryma whipplei*, the agent of Whipple's disease

Florence FENOLLAR

MD., PhD.
URMITE, UMR 7278 CNRS - INSERM - IRD
Faculté de Médecine de Marseille, FRANCE

florence.fenollar@univ-amu.fr

Tropheryma whipplei

1907 First description of Whipple's disease (Metabolic trouble)

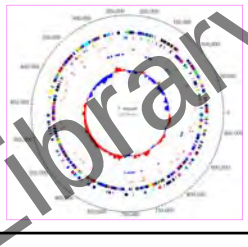
1991 Identification of the agent of Whipple's disease
A rare bacterium causing a rare chronic infection

2000 First establishment of a strain of *T. whipplei*

2003 2 full genome sequences of 2 different strains of *T. whipplei*

1 from the index strain TWIST (Cardiac valve from a Canadian patient with endocarditis)
1 from another strain TW08/27 (CSF from a German patient with a relapse of classic WD)

-Raoult D, Ogata H, Audic S, *et al. T. whipplei* twist: a human pathogenic actinobacteria with a reduced genome. Genome Res 2003;13:1800-9.
-Bentley SD, Maiwald M, Murphy LD, *et al.* Sequencing and analysis of the genome of the Whipple's disease bacterium *T. whipplei*. Lancet 2003;361:637-44.



Main characteristics *of T. whipplei* genome

- *Tw* is an Actinobacteria and presents a small genome slightly less than 1 Mbp (a 46% GC content) whereas other actinomycete genomes are from approximately 2.1–10.1 Mbp with usually a rich GC content.

- Several pathways are lacking:

These features are those of reductive genome evolution.

Tw seems to have evolved to a host-dependent organism, relying on the uptake of nutrients from outside sources.

- *Tw* carries only a few genes that regulate transcription.

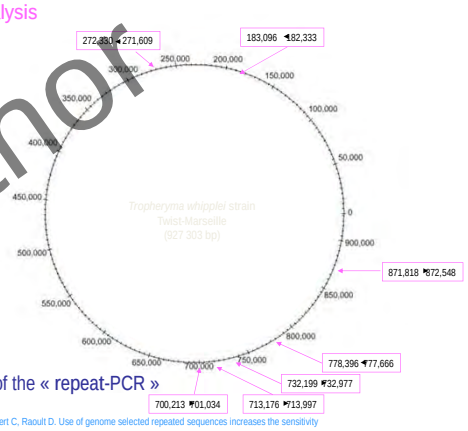
- The cell surface seems relatively abundant:

Predictions suggest that approximately 15% of all proteins are present at the cell surface.

Tw possesses many WSRs, which are surface proteins belonging to a family characterized by large size ranges and architectures.

Genome analysis

Presence of repeated sequences



Development of the « repeat-PCR »

-Fenollar F, Fournier PE, Robert C, Raoult D. Use of genome selected repeated sequences increases the sensitivity of PCR detection of *T. whipplei*. J Clin Microbiol 2004;42:401-3.

Several steps of the development of repeat-PCR

1. Specificity of *T. whipplei* repeat-DNA target *in silico* (Genbank)

2. Sensitivity of repeat-PCR *in vitro* versus classic PCR

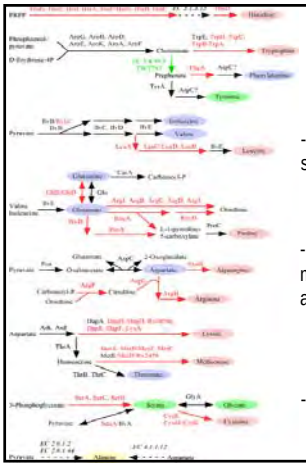
3. Specificity of repeat-PCR *in vitro* (collection of bacteria)

4. Analysis of samples from patients with Whipple's disease and from a control group tested with classic PCR and repeat-PCR

Specimens from patients	Number	Positive using classic PCR	Positive using Repeat-PCR
Total	98	33 (33.6%)	54 (55%)

All the specimens from the control group were negative.

Repeat-PCR = Our current diagnostic tool
2 PCR targeting 2 different repeated sequences should be positive to conclude to a positive assay



Genome=

Information about the metabolism of the bacterium

- Lack of numerous way for the synthesis of amino acids

- Development of a cell-free culture medium that provided all the missing amino acids

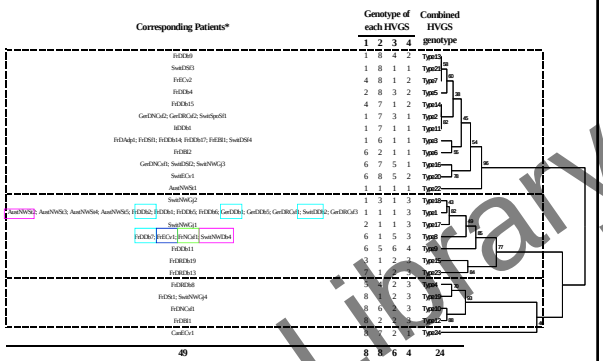
- Culture of *T. whipplei* on axenic medium

T. whipplei genotyping

-Li W, Fenollar F, Rolain JM, Fournier PE, Feurle GE, Müller C, Moos V, Marth T, Altwegg M, Calligaris-Maibach RC, Schneider T, Biagi F, La Scola B, Raoult D. Genotyping reveals a wide heterogeneity of *T. whipplei*. Microbiology 2008;154:521-7.

- Selection of 4 highly variable **sequences** from the comparison of the 2 *T. whipplei* genomes available
- Study performed on 39 DNA extracts from 39 **patients**
  - 26 classic WD with digestive involvement
  - 6 relapses of classic WD (3 digestive and 3 neurologic)
  - 5 blood culture negative endocarditis
  - 1 encephalitis
  - 1 spondylodiscitis
- 10 DNA extracts from 10 asymptomatic carriers
- Origin of the strains: France, Italy, Germany, Austria, Switzerland, Canada
- Classification in 24 different **genotypes**
- High genetic variety of *T. whipplei*

Concatenation of the 4 highly variable sequences (UPGMA tree)



Concatenation of the 4 highly variable sequences (UPGMA tree)

High genetic variety of *T. whipplei*

In this tree: 61 genotypes from 198 specimens

✱ Carriers

✳ *T. whipplei* chronic infections (classic and localized)

Cases of gastroenteritis

Genotype✱ : Same genotype detected between the family members of a same family

Sene: Senegal

T. whipplei prevalence strongly supports human transmission in homeless shelters

- Keita AK, Brouqui P, Badiaga S, Benkouiten S, Raoult D, Fenollar F. *Tropheryma whipplei* prevalence strongly supports human transmission in homeless shelters. Int J Infect Dis 2013;17:e67-8.

	Genotypes	
	3	85
2010	1	1*
2011	4	
Genotypes previously identified in France	33/125 (33.3%)	No

*Both stool and saliva specimens from the same individual present the same genotype.

	Genotypes			
	3	85	84	27
2010	1		1	1
2011		3		
Genotypes previously identified in France	33/125 (33.3%)	No	No	2/125 (1.6%)

- Suspicion of small outbreaks in this population (genotype 3 in Shelter 1 and 85 in Shelter 2 in 2011).

Predominance of *T. whipplei* genotypes 1 and 3 in central Europe

-Wetzstein N, Fenollar F, Buffet S, Moos V, Schneider T, Raoult D. EID 2013; 19:341-2.

- 191 samples from central Europe: Genetic diversity by finding 72 different *T. whipplei* genotypes
- 28.8% of all samples showed a unique genotype

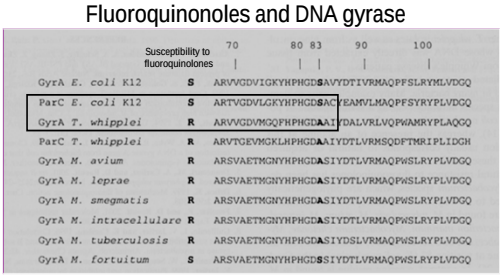
- Genotype 3:
The most common in Europe (19.9%) and could be considered epidemic and specific to France, Switzerland and Italy

- Genotype 1:
The second-most frequent genotype (15.2%) found all over central Europe (Germany: 46.2% and Austria: 80%)

Genome =

Information about genes coding for antibiotics resistance

Fluoroquinonoles and DNA gyrase



- Confirmation with *In vitro* susceptibilities:
Ofloxacin and ciprofloxacin are not effective *in vitro*

Genome=

Information about the metabolism and antibiotics resistance

Folate biosynthesis and enzymes

Dihydropteroate synthase (DHPS)
Sulfamethoxazole target

Dihydrofolate reductase (DHFR)
Trimethoprim target

- Confirmation with *In vitro* susceptibilities:

	MICs (µg/ml)		
Antibiotics	Twist	Endo 5	Slow
Sulfamethoxazole/Trimethoprim	2/0.5	4/1	4/1
Sulfamethoxazole	0.5	1	1
Trimethoprim	64	100	64
Sulfadiazine	0.5		