

Detection of a novel *Tropheryma* species in a kidney transplant recipient with nodular pulmonary infiltrates

Anne Vankeerberghen, PhD
OLV-hospital Aalst
Belgium

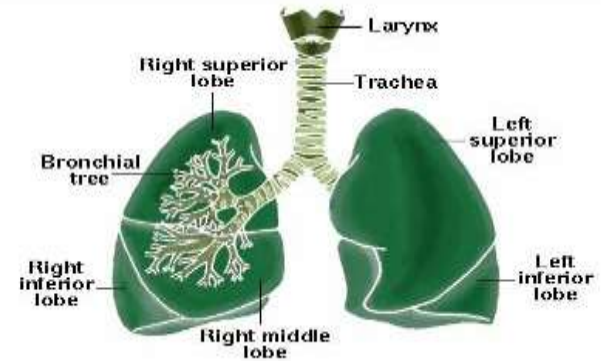
27th
ECCMID

Vienna, Austria
22 – 25 April 2017



Case report

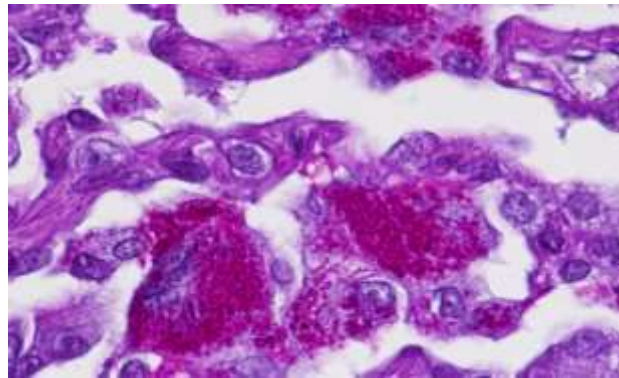
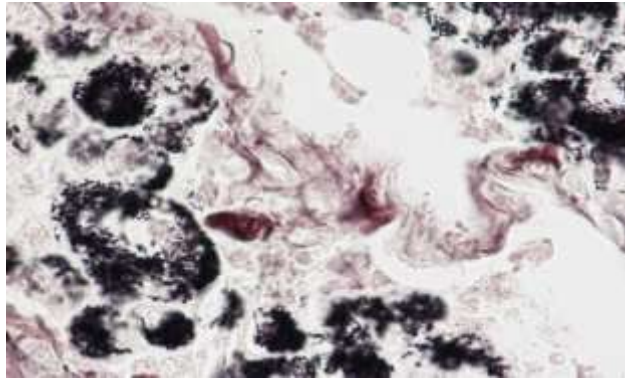
- Female patient, 40 years old, lupus nephritis
- Kidney transplantation: 2013
- End 2014: sustained increased CRP
pulmonary problems
recurrent pleurisy
multiple nodular infiltrates
opacification
resp. sample: culture / PCR negative
- 02/2015: open lung biopsy left lower lobe



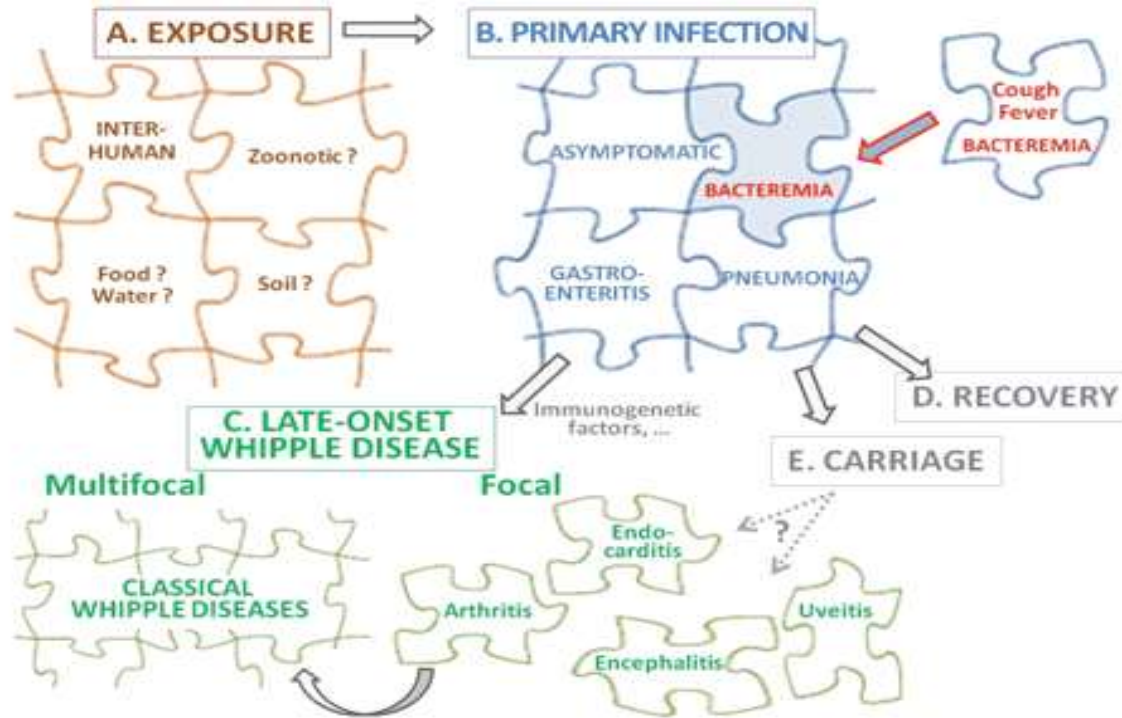
<https://svbiologie.wikispaces.com/Ademhaling>

Case report

- Lung biopsy left lower lung : Pathology: FFPE
- Gram staining: negative
- Acid fast staining: negative : no mycobacteria
- Grocott and PAS staining: positive : suggestive for *Tropheryma whipplei*



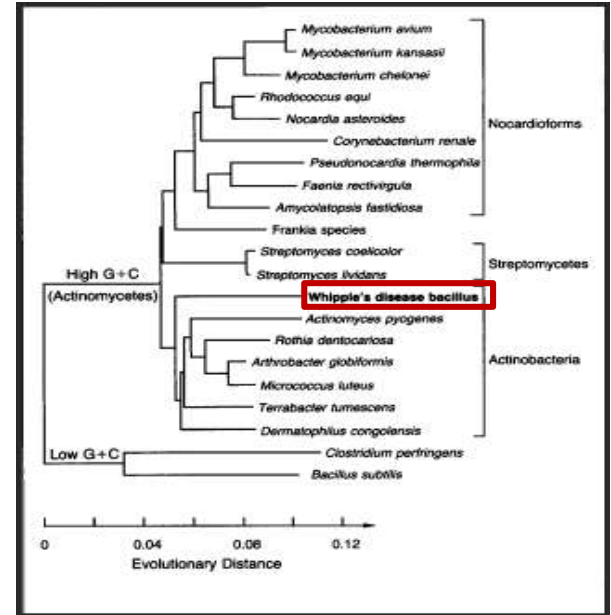
Whipple's disease



G. Greub, CID 2010; 51(5):522-524

Tropheryma whipplei

- 1907: George H Whipple: Whipple's disease
- 1949: PAS staining of macrophages of infected individuals
- 1952: Antibiotic therapy
- 1991: Wilson et al: 16SrRNA sequencing
- 1992: Relman et al: 16SrRNA sequencing:
Tropheryma whippelii



Relman et al., The New England Journal of Medicine 1992, Vol 327, p293-301

Tropheryma whipplei

- 1997: Schoedon et al: growth in vitro in macrophages
- 2001: La Scola et al: stable subculture and characterization: *Tropheryma whipplei*
- 2003: Raoult et al - Bentley et al: whole genome sequencing
- 2004: Fennolar et al: real time PCR (specific repetitive sequence element): specific molecular detection

Tropheryma whipplei

Scientific classification

Kingdom: Bacteria
Phylum: Actinobacteria
Class: Actinobacteria
Subclass: Actinobacteridae
Order: Actinomycetales
Family: Cellulomonadaceae
Genus: Tropheryma
Species: ***T. whipplei***

Binomial name

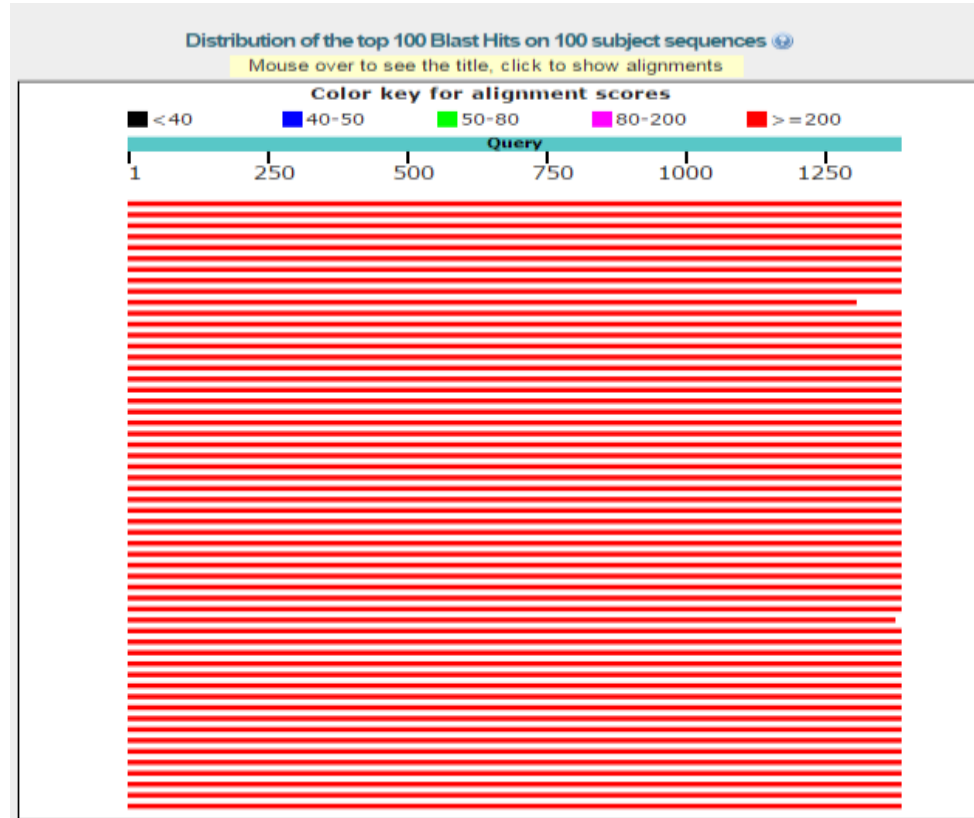
Tropheryma whipplei

La Scola et al. 2001

Lung biopsy: molecular diagnosis

- DNA extraction FFPE sample: FFPE extraction kit (Qiagen)
- ITS fungal PCR: negative
- *T. whipplei* real time PCR: (non-coding repeat; Fenollar et al, 2004): **negative**
- 16S rRNA sequence (short analysis and blast ncbi; greengenes): ***T.whipplei***
but only 96% (288/300) agreement!

Blast analysis 16S rRNA complete sequence



Blast analysis 16S rRNA complete sequence

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected:0

- [Alignments](#) [Download](#) [GenBank](#) [Graphics](#) [Distance tree of results](#)
- [Tropheryma whipplei strain Twist 16S ribosomal RNA gene, complete sequence](#)
- [Tropheryma whippelii 16S rRNA gene \(partial\), IGS and 23S rRNA gene \(partial\)](#)
- [Tropheryma whipplei str. Twist, complete genome](#)
- [Tropheryma whipplei strain Twist-Marseille 16S ribosomal RNA gene, partial sequence](#)
- [Tropheryma whippelii 16S rRNA, 23S rRNA, 5S rRNA genes and IGS, isolated from patient 1](#)
- [Tropheryma whippelii 16S ribosomal RNA gene, partial sequence](#)
- [Tropheryma whipplei TW08/27, complete genome: segment 3/3](#)
- [Tropheryma whippelii from patient 3 ribosomal RNA operon, complete sequence](#)
- [Tropheryma whippelii from patient 2 ribosomal RNA operon, complete sequence](#)
- [Tropheryma whippelii homologue to Escherichia coli 16S rRNA \(from bp 28 - 1370\) gene](#)
- [Uncultured bacterium clone 10-1184 16S ribosomal RNA gene, partial sequence](#)
- [Diaminobutyricibacter tongyongensis strain KIS66-7 16S ribosomal RNA, partial sequence](#)

Max score	Total score	Query cover	E value	Ident	Accession
2363	2363	100%	0.0	98%	NR_074674.1
2363	2363	100%	0.0	98%	AJ551273.1
2363	2363	100%	0.0	98%	AE014184.1
2363	2363	100%	0.0	98%	NR_027540.1
2363	2363	100%	0.0	98%	X99636.2
2363	2363	100%	0.0	98%	AF202891.1
2358	2358	100%	0.0	98%	BX251412.1
2358	2358	100%	0.0	98%	AF190688.1
2358	2358	100%	0.0	98%	AF190687.1
2215	2215	94%	0.0	98%	M87484.1
2024	2024	100%	0.0	93%	KC554563.1
2024	2024	100%	0.0	93%	NR_134019.1
2003	2003	100%	0.0	92%	LT223676.1
1999	1999	100%	0.0	92%	LT558829.1
1999	1999	100%	0.0	92%	NR_109609.1
1999	1999	100%	0.0	92%	EU086820.1

Best hit: *Tropheryma whipplei* but only 98%

- [Pseudoclavibacter alba partial 16S rRNA gene, strain Marseille-P2081](#)
- [Pseudoclavibacter alba partial 16S rRNA gene, strain Marseille-P2081](#)
- [Rudaibacter terrae strain 5GHs34-4 16S ribosomal RNA gene, partial sequence](#)
- [Pseudoclavibacter sp. G665 16S ribosomal RNA gene, partial sequence](#)

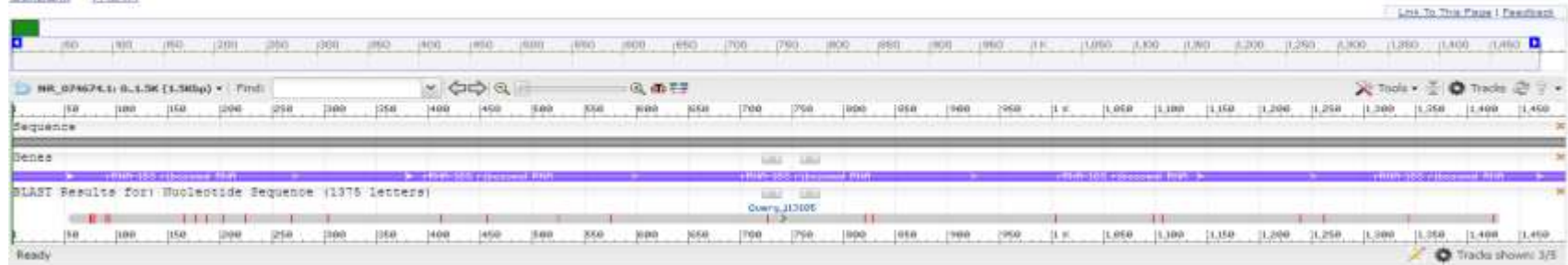


Blast analysis 16S rRNA complete sequence

Tropheryma whipplei strain Twist 16S ribosomal RNA gene, complete sequence

NCBI Reference Sequence: NR_074674.1

[GenBank](#) [FASTA](#)



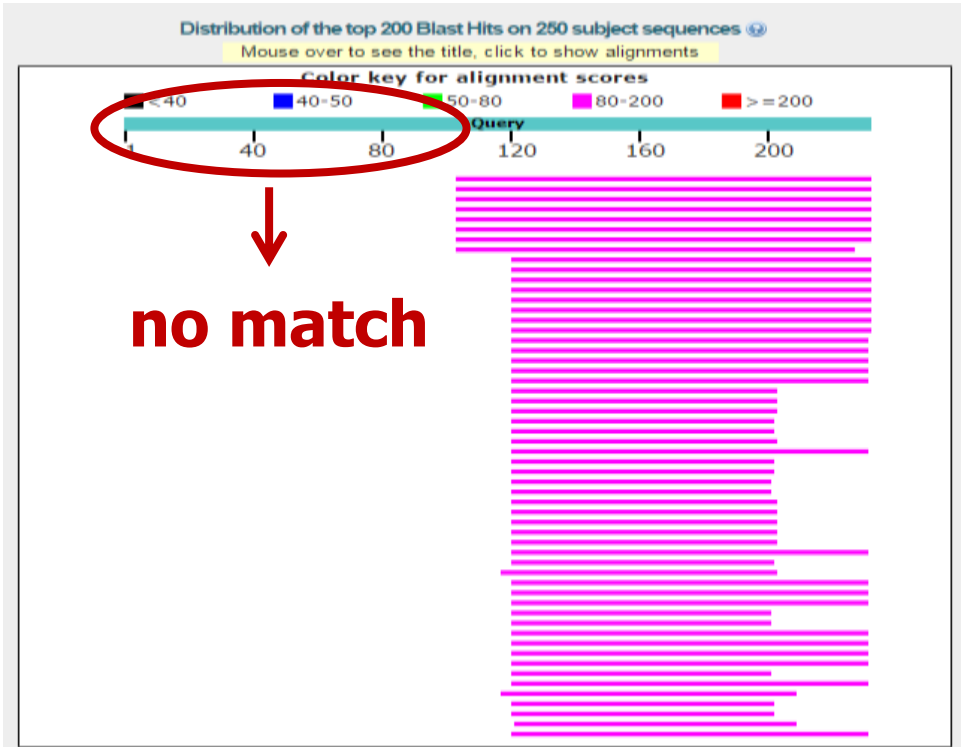
**26 mismatches spread over the 1375 bp
16S rRNA gene fragment**

23S rRNA sequence analysis

- Tropheryma: Actinomycetales
- Actinobacteria: insertion (compared to *E. coli*) in the hypervariable domain III of the 23S rRNA gene: species differentiation
- Insertion of *T. whipplei*
 - secondary structure similar to other actinobacteria
 - smaller (80 nt long) compared to other actinobacteria (86-116 nt long)
 - unique, contains no “known” group-specific signatures
- 23S rRNA gene fragment was amplified and sequenced (primers from Roller et al 1992; Hinrikson et al 2000).

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La Scola et al. 2001

Blast analysis 23S rRNA : 230 bp fragment



Blast analysis 23S rRNA : 230 bp fragment

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected: 0

Alignments [Download](#) [GenBank](#) [Graphics](#) [Distance trees of results](#)

<input type="checkbox"/>	Tropheryma whipplei strain Twist 23S ribosomal RNA gene, complete sequence
<input type="checkbox"/>	Tropheryma whipplei str. Twist, complete genome
<input type="checkbox"/>	Tropheryma whipplei TW08/27, complete genome; segment 3/3
<input type="checkbox"/>	Tropheryma whippelli from patient 3 ribosomal RNA operon, complete sequence
<input type="checkbox"/>	Tropheryma whippelli from patient 2 ribosomal RNA operon, complete sequence
<input type="checkbox"/>	Tropheryma whippelli 16S rRNA, 23S rRNA, 5S rRNA genes and IGS, isolated from patient 1
<input type="checkbox"/>	Tropheryma whippelli 23S ribosomal RNA gene, partial sequence
<input type="checkbox"/>	Tropheryma whipplei str. Twist strain TWIST Marseille 16S ribosomal RNA gene, partial sequen
<input type="checkbox"/>	Leifsonia sp. 98AMF genome assembly, chromosome.1
<input type="checkbox"/>	Leifsonia sp. 467MF genome assembly, chromosome.1
<input type="checkbox"/>	Leifsonia sp. 509MF genome assembly, chromosome.1
<input type="checkbox"/>	Leifsonia sp. 197AMF genome assembly, chromosome.1
<input type="checkbox"/>	Leifsonia xvii strain SE134, complete genome
<input type="checkbox"/>	Leifsonia sp. 21MFCrub1.1 genome assembly, chromosome.1

Max score	Total score	Query cover	E value	Ident	Accession
179	179	55%	2e-41	91%	NR_076189.1
179	179	55%	2e-41	91%	AE014184.1
179	179	55%	e-41	91%	GX251412.1
179	179	55%	e-41	91%	AF190688.1
179	179	55%	e-41	91%	AF190687.1
179	179	55%	2e-41	91%	X99636.2
179	179	55%	2e-41	91%	AF148136.1
176	176	53%	2e-40	82%	AH010582.2
131	131	48%	9e-27	86%	LT630032.1
131	131	48%	9e-27	86%	LT630002.1
131	131	48%	9e-27	86%	LT630707.1
131	131	48%	9e-27	86%	LT629694.1
131	131	48%	9e-27	86%	CF014751.1
125	125	48%	4e-25	86%	LT629867.1

Best hit: *Tropheryma whipplei* but only 91%



Conclusion

- PAS staining: positive
- *T. whipplei* specific PCR (rep seq): negative
- 16S rRNA sequence analysis:
 - Best match: *T. whipplei*
 - but only 98% similarity
- 23S rRNA sequence analysis:
 - Best match: *T. whipplei*
 - but only 91% similarity
 - and no match for the 5' 102 bp

Do we have a new *Tropheryma* species?



Further identification is needed



Limitation: sample is FFPE lung biopsy



No culture possible

What about the patient ?

- Kidney transplant patient with recurrent pleurisy and multiple nodular infiltrates
- 19/03/2015: open lung biopsy left lower lung: “*T. whipplei*-like”
- 30/06/2015: clinically worse
chest pain (breathing)
subfebrile
CRP 14,8 mg/L
- Consult microbiology: start antibiotic therapy **as for *T. whipplei*** :
ceftriaxone iv (14 days) +
co-trimoxazole po (long term)

What about the patient ?

- Start antibiotic therapy: 30/06/2015
- 03/08/2015: CRP<0,6 mg/L; no chest pain or fever
- 31/08/2015: CT thorax: less opacification
less radiating strands
- 19/10/2015: change antibiotic therapy (granulocytopenia):
from co-trimoxazole to doxycycline (long term)
- 04/07/2016: CRP 0,7 mg/L
stop therapy
- 20/03/2017: no complaints, CRP 0,9 mg/L
- Lifetime follow-up is proposed

Antibiotic therapy as for *T. whipplei* seemed successful

What about the lab ?

Design of a new triplex real time PCR for the detection of *Tropheryma* species (including *T. whipplei*) in clinical samples.

Targets:

- Internal extraction and inhibition control
- Repeat sequence of *Tropheryma whipplei*
- 16S sequence of *Tropheryma* species

Thank you!

Technicians Molecular Biology Lab: Magda, Elfi, Inge, Astrid and Melissa

Microbiologists: Stijn Jonckheere, Hans de Beenhouwer, Kristien Van Vaerenbergh and An Boel

Pathologist: Hendrik De Raeve

Nephrologist: Rogier Caluwe

