

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Raoult D, Fenollar F, Birg M-L. Culture of *T. whipplei* from the stool of a patient with Whipple's disease. N Engl J Med 2006;355:1503-5.

Supplementary Information Regarding Figure 1.

Real-time quantitative PCRs were performed on control cultures of *T. whipplei*, isolates made from one gram of feces from a patient with Whipple's disease, and isolates made from one gram fecal samples from 3 healthy people (A,B,C) to which 10^5 , 10^4 or 10^3 *T. whipplei* Twist per ml (determined by quantitative PCR (5)) had been added. The stool samples were suspended in Rinaldini medium (NaCl, 6.8 g; KCl, 0.4 g; NaH_2PO_4 , 0.15 g; glucose, 1.0 g; NaCOH_3 , 2.2 g; phenol red, 0.002 g; in 1 ml of distilled water) and mixed v/v with glutaraldehyde 2% (Sigma, St Quentin-Fallavier, France) diluted in sodium bicarbonate pH8. After vortexing for 5 minutes and centrifugation at 7.500 g for 10 minutes, the pellet was harvested, washed twice in water and incubated in 5 ml of SAM containing ciprofloxacin (1 $\mu\text{g}/\text{ml}$) and amphotericin B (2 $\mu\text{g}/\text{ml}$) in 25 cm^2 flasks held at 37°C in a 5% CO_2 atmosphere (5). Each month, 50% of the SAM was replaced; for the first three months, both ciprofloxacin (1 $\mu\text{g}/\text{ml}$) and amphotericin B (2 $\mu\text{g}/\text{ml}$) were added and thereafter only amphotericin B (2 $\mu\text{g}/\text{ml}$) was added to the SAM. Growth kinetics were monitored monthly by immunofluorescence (supplementary data 2) and quantitative real-time PCR (5). For the PCR, samples were submitted to DNA extraction using QIAamp DNA MiniKit (Qiagen, Hilden, German) according to manufacturer recommendations. This PCR reaction, which targeted a 105 bp repeated sequence of the bacterium, incorporated the primer pair TW27F (5'TGTTTTGTA CTGCTTGTAACAGGATCT) and TW182R (5'TCCTGCTCTATCCCTCCTATCATC), and a Taqman* probe (27F-182R, 6-FAM-AGAGATACATTTGTGTTAGTTGTTACA-TAMRA) to the reaction mix. PCR was done in a LightCycler instrument (Roche biochemicals, Mannheim, Germany). The PCR mixture included a final volume of 20 μl with 10 μl of the Probe Master kit (QIAGEN, Hilden, Germany), 0.5 μl (10 pmol/ μl) of each primer, 5 μl (2 $\mu\text{mol}/\mu\text{l}$) of probe, 3 μl of distilled water, and 2 μl of extracted DNA. The amplification conditions were as follows: an initial denaturation step at 95°C for 15 minutes, followed by 40 cycles of denaturation at 95°C, annealing and elongation at 60°C for 60 seconds, with fluorescence acquisition in single mode. Every 5 samples, negative controls (sterile distilled water, PCR mix and biopsy samples from healthy humans) were inserted.

Supplementary data 1.

Confirmation of the purity of the culture.

We have performed broad range 16S rDNA PCR using the primers 536F (5'CAGCAGCCGCGGTAATAC) and rp2 (5'ACGGCTACCTTGTTACGACTT) on the isolated strain. The obtained sequence from the amplified product was blasted on GenBank and was 100% homologous with the 16S rRNA sequence of *T. whipplei* (Accession number: AF251035.1.). Several drops of the culture medium infected with *T. whipplei* were inoculated on chocolate and blood agar and cultured under aerobic and anaerobic conditions for seven days. No growth of any other bacteria was observed. Based on these data, we consider the culture of *T. whipplei* from the stools as pure.

Exclusion of pitfalls of intra-laboratory contamination.

We have applied the multi-spacer typing method for *T. whipplei* genotyping. We have targeted a 214-bp sequence from an intergenic spacer localized between the ORFs 133 and 134 of *T. whipplei* genome using the primers TWT133F (5'GCTGCGCGAAGTAATTTG) and TWT133R (5'AGATACATGCGGAGATACT) and a 219-bp sequence from an intergenic spacer localized between the gene SecA and the ORF 131 of *T. whipplei* genome using the primers SECA F (5'TTTGTCATAGGCATTTCTGTAG) and SECA R (5'AGACCTCACTGTTATACGGAT). Genotyping has been performed for all the *T. whipplei* strains isolated in our laboratory, for the patient's stools and for the isolated strain from the stools. Based on the sequences of the intergenic spacers, the strain of *T. whipplei* from our patient is unique in comparison to all

other strains isolated in our laboratory. The genotyping from the patient's stools and the isolated strain are the same. Thus, we can exclude a problem of intra-laboratory contamination.

Confirmation of the establishment of the strain.

During the propagation step, we froze aliquots of *T. whipplei* at -80°C . We have tested one of our frozen aliquots obtained from the third subculture using specific real-time quantitative PCR. One of the aliquot from the third subculture has been tested using *T. whipplei* real-time quantitative PCR. The PCR was positive, allowing the detection of 10^5 DNA copies of *T. whipplei* in $10\mu\text{l}$ of the frozen aliquot confirming the establishment of our strain.

Supplementary data 2.

Positive immunofluorescence of control culture of the stools of the patient performed the eleventh month using specific anti-*T. whipplei* rabbit polyclonal antibodies.

