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Molecular basis of *Tropheryma whipplei* doxycycline susceptibility examined by transcriptional profiling

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***Objectives and methods*: *Tropheryma whipplei* is a poorly studied bacterium responsible for Whipple's disease. In this study, its susceptibility to doxycycline was investigated at a transcriptional level using a whole-genome DNA microarray.**

***Results*: Exposure of *T. whipplei* to the MIC of doxycycline (0.5 mg/L) induced antibiotic-specific primary expression profiles, while indirect effects were detected at 10× MIC. In contrast to what was observed for several microorganisms exposed to antibiotics, the heat-shock proteins were not affected. Consistent with the mode of action of this translation inhibitor, genes encoding for ribosomal proteins and translation factors were differentially transcribed. This analysis also evidenced the regulation of genes that should account for cell growth arrest. Long-term survival of non-replicating bacteria is likely to be ensured by an increased level of ppGpp, the nucleotide effector of the stringent response. The gene expression profile observed with 10× MIC was mainly characterized by the up-regulation of ABC transporters that possibly form efflux and detoxification systems, through which *T. whipplei* may limit the effects of this bacteriostatic compound. Obtained microarray data showed good agreement with real-time quantitative PCR (R^2 = 0.969).**

***Conclusions*: This work represents the first comprehensive genomic approach providing insights into the expression signature triggered by the exposure of *T. whipplei* to antibiotics.**

Keywords: antibiotics, transcription, efflux pumps, microarrays

Introduction

Tropheryma whipplei is a facultative intracellular bacterium, responsible for Whipple's disease,[1] a chronic multisystemic disorder mainly characterized by intestinal malabsorption but which can involve other organs such as the heart or central nervous system.[2] Because of its heterogeneous clinical presentation associated with the lack of gastrointestinal symptoms for approximately 15% of patients,[3] Whipple's disease is still frequently misdiagnosed[4,5] and fatal if untreated.[6,7] While antibiotics are mandatory to cure Whipple's disease, the optimum antimicrobial therapy remains controversial with respect to both the choice of drug and the duration of treatment.[7] The current reference treatment was determined empirically on the basis of only a few clinical observations. The efficiency of trimethoprim/sulfamethoxazole was initially evidenced on a patient with allergy to penicillin and intolerance to tetracycline.[8] Daily treatment with penicillin G (6–24 million U iv) plus streptomycin (1 g im) or the third-generation cephalosporin (e.g. ceftriaxone 2 g iv) for a period of 2 weeks, followed by oral co-trimoxazole (trimethoprim/sulfamethoxazole 160 mg/800 mg) twice daily for at least 1 year is actually recommended.[9] In the case of sulphonamide intolerance, doxycycline (100 mg) twice daily has been proposed as an alternative choice.[4]

The establishment of the first clinical isolate of *T. whipplei*[10] has opened new perspectives into the diagnosis and treatment of Whipple's disease. This finding allowed a formal evaluation of a selection of compounds aimed at a better antimicrobial strategy. The susceptibility of *T. whipplei* was evaluated *in vitro* using both bacteria grown in MRC-5 cells[11,12] or under axenic conditions.[13] Although most antibiotics were active, doxycycline was shown to be particularly effective.[12,13] Since *T. whipplei*

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survives in acidic vacuoles,[14] alkalinization by lysosomotropic agents was hypothesized to be crucial for the bactericidal activity of this compound, as is the case for *Coxiella burnetii*, another bacterium intracellularly located in such vacuoles.[15] The combination of doxycycline and hydroxychloroquine effectively killed *T. whipplei* and was proposed as a possible treatment to eradicate such intracellular microorganisms.[12,13]

A major achievement resulting from the cultivation of this poorly studied pathogen was the completion of genome sequencing.[16,17] Several concrete post-genomic applications were delineated from the exploration of these data[18] including the design of a comprehensive culture medium allowing *T. whipplei* to grow axenically[19] and the optimization of RNA extraction.[18] An immunoproteomic approach was also investigated through bi-dimensional gel analysis either coupled to mass spectrometry or to Western-blotting.[20] The successful transcriptional analysis of *T. whipplei* exposed to various temperatures was recently achieved using a dedicated whole DNA microarray.[21] This latter application now offers the opportunity to dissect the genome-wide expression profiling of this bacterium within various experimental conditions, including antibiotic exposure.[22]

Doxycycline is a semi-synthetic structural isomer of the tetracycline family that exhibits a bacteriostatic activity on many bacteria.[23] The main mechanism of tetracycline action is known to be the inhibition of protein synthesis through binding to the bacterial ribosomal 30S subunit.[23,24] Secondary effects also contribute to bacterial inhibition.[25] The understanding of the precise mode of action of antibiotics will in time help to devise appropriate therapeutic strategies. In this respect, we have explored the potential of using gene expression profiling to dissect the molecular basis responsible for *T. whipplei* susceptibility to doxycycline. This was performed starting from bacteria grown under axenic conditions and using a new RNA amplification strategy (M. V. La, P. Francois, C. Rovery, S. Robineau, P. Barbry, J. Schrenzel, D. Raoult and P. Renesto, unpublished results) to reduce the amount of required material.

Materials and methods

Bacterial strain, growth conditions and RNA extraction

T. whipplei strain Twist[1] was grown under axenic conditions in DMEM/F12 medium (Invitrogen Life Technologies, Carlsbad, USA) supplemented with 10% fetal calf serum, 1% L-glutamine and 1% human non-essential amino acids (Invitrogen) as previously described.[19] Mid-log cultures (12 days old) were further incubated or not (control) for 6 h with two different concentrations of doxycycline (Vibravenosa, Pfizer), i.e. MIC (0.5 mg/L) and 10× MIC (5 mg/L), respectively.[13] After the antibiotic treatment, bacteria (200 mL) were harvested by centrifugation 16 900 **g** for 10 min at 4°C. Resulting pellets were immediately sonicated in 1 mL of Trizol® Reagent (Invitrogen Life Technologies) and frozen in liquid nitrogen before storage at −80°C. RNA extraction was carried out according to the reference protocol,[18] eventual DNA contamination being removed by the incubation of obtained samples with RNase-Free DNase Set (QIAGEN) followed by a clean-up step on RNeasy column (QIAGEN). Amount and quality of each RNA sample was monitored using the Bioanalyzer 2100 with RNA Nano LabChips™ (Agilent, Palo Alto, CA, USA). Moreover, the absence of DNA contamination was assessed by reverse transcriptional and standard PCR, as previously described.[21] Starting from 200 mL of *T. whipplei* culture, the amount of recovered RNA varied from 0.385 to 1.918 μg. For each condition, RNA was extracted from at least three independent bacterial cultures.

cDNA synthesis, amplification, fluorescent labelling and hybridization

RNA (500 ng) was used to synthesize cDNA with reverse transcriptase (Superscript II, Invitrogen, Carlsbad, CA, USA). Obtained cDNA (20 ng) was then amplified using the Genomiphi DNA amplification kit (Amersham Biosciences, Piscataway, NJ, USA) and random primers before labelling with the BioPrime DNA Labeling System (Invitrogen, Carlsbad, CA, USA). The levels of Cy3 and Cy5 incorporation were quantified by absorbance measurement at 550 and 650 nm, and samples processed for hybridization on microarray when incorporation levels were ≥50 pmol of fluorochromes per μg of DNA.

Microarrays spotted with 804 PCR amplicons corresponding to 99.5% of *T. whipplei* were used in this study.[21] Briefly, following a post-processing step,[21] hybridization was carried out using two DNA samples (7 μg each) that were labelled either with Cy3- or with Cy5-dCTP. Following the fluorochrome incorporation, samples were pooled, evaporated (SpeedVac™ concentrator) and finally resuspended in 6 μL of nuclease-free H_2O. The mixture was heated at 95°C for 2 min and cooled on ice for 30 s before addition of 7.5 μL of microarray hybridization buffer (supplied in the CyScribe kit; Amersham) and 15 μL of 100% (v/v) formamide. The sample was applied to the microarray slide by covering a 24 × 60 mm glass coverslip. Following 18 h of incubation at 42°C, the slide was washed then dried with compressed nitrogen, and then scanned with ScanArray®Express (Perkin Elmer, Boston, MA, USA). Laser power was set to 100% and photomultiplicator (PMT) power was set between 55% and 75%, depending on the slides.

Microarray data analysis

TIF images containing the data from each fluorescence channel were quantified with the QuantArray® Microarray Analysis Software version 3.0.0.0 (Packard BioScience) to obtain the signal intensity and local background of each spot, and to exclude preliminarily irrelevant values, as flagged. The data filtering and normalization were then processed with the Microsoft Excel software. Spots with background-corrected signal intensity (median) in both channels lower than twice the background intensity (median) were discarded from further analysis. The background-subtracted signals derived from the remaining spots were normalized by total intensity normalization methods and then the normalized log ratio of test/reference signal for each spot was recorded.

Because the incorporation of Cy3 and Cy5 dyes into DNA can differ significantly, instead of using dye-swap, we always labelled control and experimental samples with Cy3 and Cy5, respectively, such a procedure being recommended for the design of microarray experiments.[26] In addition, three hybridizations of Cy3-labelled control versus Cy5-labelled control were performed. Moreover, to improve the reproducibility of quantification, all experiments were conducted in triplicate, yielding 12 independent measurements for each condition (representing four

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technical and three biological experiments). Gene expression was determined as the mean of the 12 values obtained. The data analysis was processed using TMEV software (http://www.tm4.org/). ANOVA test was applied to the data and genes with a P value <0.05 were considered to have significant differential expression. Significant changes in gene expression were identified with significance analysis for microarrays (SAM) using two-class unpaired and a 1.5-fold cut-off.

Real-time quantitative PCR analysis

To validate the results obtained from microarray, gene expression levels were measured by real-time quantitative PCR (qPCR) starting from 12.5 ng of amplified *T. whipplei* cDNA as template. The reaction was performed with the LightCycler system (Roche, Basel, Switzerland) and SYBR green master mix, according to the manufacturer's instructions. For each pair of primers [see Table S1, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)], a standard curve was made with genomic DNA of *T. whipplei*. Each gene was assayed in duplicate and the relative expression ratios of target genes were calculated using the Pfaffl model.[27]

Accession number

All results are available in the GEO database (http://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE5717.

Results

Microarray experimental design and validation of data

The transcriptional profiling of *T. whipplei* (Twist strain) exposed to doxycycline was performed using whole genome microarrays that we previously validated.[21] In our experimental design, the control group corresponded to the case when bacteria were not exposed to antibiotics while the test groups corresponded to a 6 h exposure to either the 1× MIC or to the 10× MIC of doxycycline. This MIC value (0.5 mg/L) was determined in our laboratory on bacteria grown under similar culture conditions and using a real-time quantitative PCR assay.[13] To circumvent the difficulty in obtaining high yield and quality of RNA from this slow growing bacterium, the cDNA was amplified with random nucleotides, a powerful strategy successfully devised for the global transcriptome analysis of *Rickettsia conorii* (M. V. La, P. Francois, C. Rovery, S. Robineau, P. Barbry, J. Schrenzel, D. Raoult and P. Renesto, unpublished results). Quantification and normalization of data obtained were processed as previously described.[21] ANOVA and SAM test were applied to determine the genes at a significant level of confidence of above 95% with a 1.5-fold cut-off. Microarray data were confirmed by real-time qPCR performed on 12 targets found to be up-regulated, down-regulated or unchanged and using amplified cDNA of *T. whipplei* as template. When comparing both methods, a high correlation coefficient ($R^2 = 0.969$) and a slope of 1.1827 were observed (Figure 1).

Global analysis of genes differentially expressed in T. whipplei exposed to doxycycline

The transcriptome analysis of *T. whipplei* in response to doxycycline revealed the differential expression of 81 genes (10.1%) with 1× MIC and 59 genes (7.3%) with 10× MIC [Table S2, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. Only 14 genes were found to be regulated under both experimental conditions. For these 14 genes, the observed fold changes were almost identical. Other changes were specific for the concentration of antibiotic used. The degree of differential expression ranged from 4.91-fold up-regulated to 2.15-fold down-regulated. Over the entire experiments, more genes were up-regulated than down-regulated (5.6% versus 4.5% for 1× MIC; and 5.3% versus 2.0% for 10× MIC). The percentages of these genes classified according to their functional categories are expressed as pie charts in Figure 2. Of the genes differentially transcribed in response to doxycycline, the higher proportion encodes proteins with unknown function. The genes commonly involved in adaptation to environmental changes, including the heat shock proteins (*dnaK* and *groE* operon) and ATP-dependent protease (Clp and Lon)[28] were not modified by doxycycline. Exposure of *T. whipplei* to this antibiotic mainly altered the transcription of genes involved in translation, in nucleotide metabolism and in membrane transport.

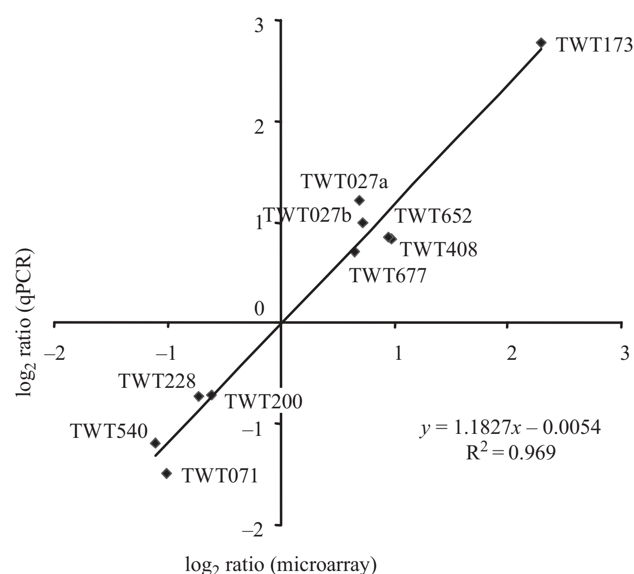

Figure 1. Validation of microarray-based expression profiles by real-time qPCR. The relative transcriptional levels for 12 genes, namely *purE*, *rpoB*, *ftsX*, *murG*, *rplF*, *rpsL*, *phoT*, *atpH*, TWT126, TWT173, TWT408 and TWT652, were determined by real-time qPCR. Following normalization of data based on values measured with non-regulated transcripts (TWT126, *phoT* and *atpH*), the real-time qPCR $\log_2$ values were plotted against the microarray $\log_2$ values. Since the transcript level of TWT027 was up-regulated with the two concentrations of doxycycline used in this study, real-time qPCR was performed using both cDNAs as template; indicated as TWT027a (1× MIC) and TWT027b (10× MIC). The correlation coefficient (R^2) for comparison of the two datasets was 0.969.

Altered expression of genes involved in translation

As expected, the transcriptomic profiles of genes related to translation were significantly affected by doxycycline. These genes essentially encode for either 30S or 50S ribosomal proteins. Only two genes (*rplF* and *pth*) were found to be modified to the same extent with both antibiotic concentrations. Overall, the genes implicated in translation were overexpressed when

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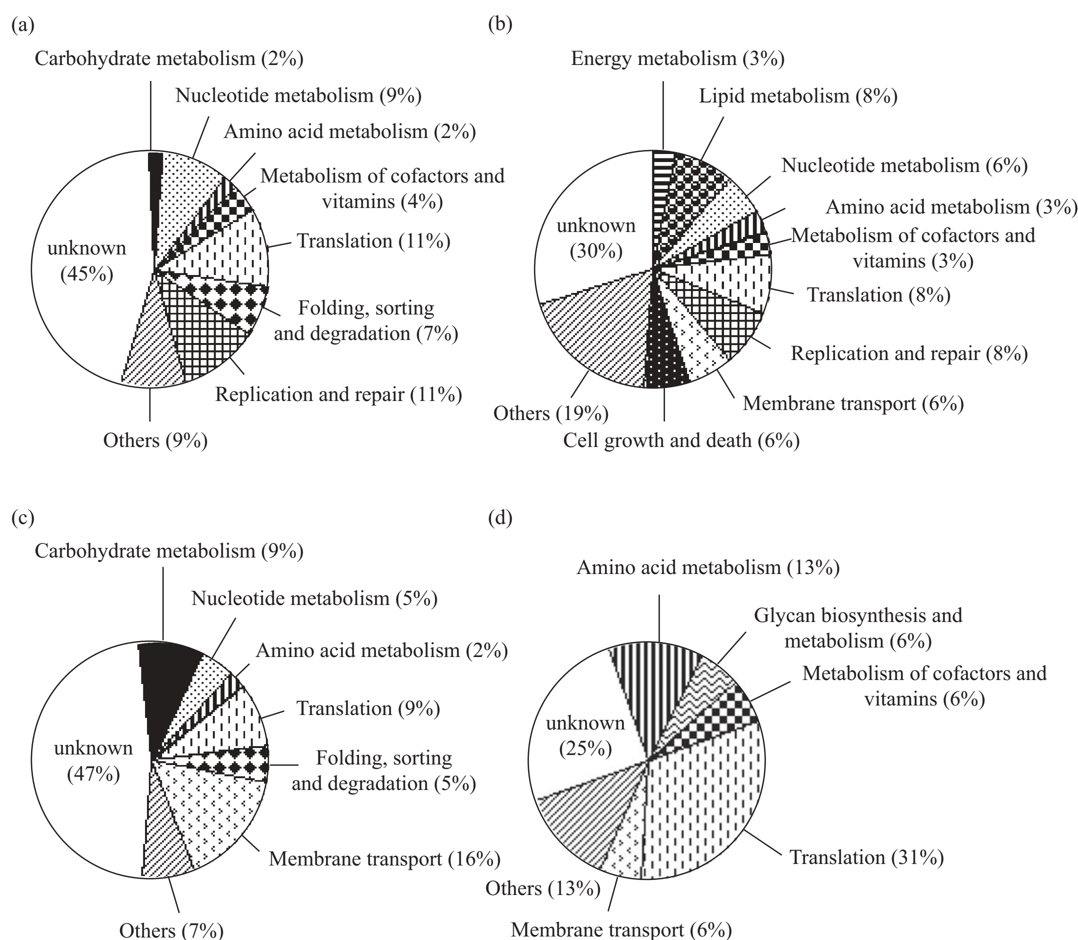

Figure 2. Distribution of *T. whipplei* genes regulated upon doxycycline exposure. The percentages of up-regulated (a and c) and down-regulated (b and d) transcripts classified according to their respective functional categories were calculated by comparing the transcriptional profile of *T. whipplei* incubated with either 1× MIC doxycycline (a and b) or 10× MIC doxycycline (c and d) with that of untreated cells. The numbers of modified transcripts were 45, 36, 43 and 16 for (a), (b), (c) and (d), respectively.

bacteria were grown in the presence of a sub-lethal concentration of doxycycline while the reverse was observed when the drug concentration was increased.

Regulation of genes involved in nucleotide metabolism

Doxycycline up-regulated the transcription of several genes involved in the purine metabolism pathway including *purE*, *purK* [EC:4.1.1.21] and *purF* [EC:2.4.2.14]. Such an up-regulation was also observed for the *relA* gene encoding GTP pyrophosphokinase [EC:2.7.6.5] implicated in guanosine-tetraphosphate (ppGpp) synthesis. In contrast to *purK* and *purE*, which are adjacently located on the *T. whipplei* genome and susceptible to both antibiotic concentrations, *purF* and *relA* were exclusively regulated by the 1× MIC of doxycycline. Under this experimental condition, we also observed a down-regulation of genes involved in RNA synthesis (i.e. *rpoB* and *rpoA*).

Regulation of genes encoding membrane transport proteins

This category of genes was almost exclusively differentially regulated upon 10× MIC. Under this condition, six open reading frames (ORFs) with similarity to ABC transporters were overexpressed including TWT173 and TWT175. In this respect, we also noticed that two unknown function genes (TWT174 and TWT176) respectively located at position 221 724 through position 222 617 and at position 223 413 through position 223 991 in the *T. whipplei* Twist genome were co-transcribed along with TWT173 and TWT175 and presumably form the ABC transporter operon (Figure 3).

Other changes associated with doxycycline exposure

A number of genes involved in DNA repair, recombination and replication were targeted by doxycycline. We also observed several changes in lipid and carbohydrate metabolism. Concerning the latter, the up-regulation of gene transcripts was mainly detected with the 10× MIC of doxycycline.

Discussion

Overview of the observed transcriptional changes

The aim of this study was to dissect the effect of the MIC of doxycycline (1× MIC) on *T. whipplei* using DNA microarrays.

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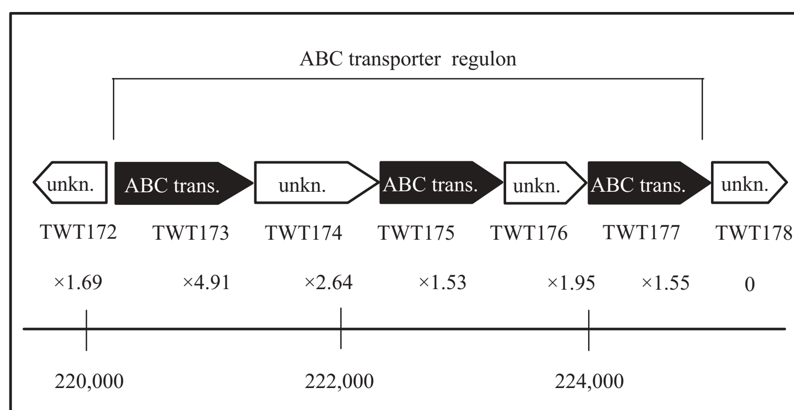

Figure 3. Schematic representation of a putative ABC transporter regulon within the *T. whipplei* genome. Values indicate the fold-change in transcript amounts for each gene determined from *T. whipplei* exposed to 10× MIC doxycycline for 6 h. White boxes correspond to ORFs encoding proteins with still unknown functions. unkn., unknown; ABC trans., ABC transporter.

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Considering both the replication time of this bacterium under axenic conditions[19] and the time course of its global RNA expression profile response following thermal shocks,[21] the incubation time was fixed at 6 h. The percentage of transcript changes detected in response to such a treatment (10.1%) is consistent with data described for other translation-inhibiting antibiotics.[29–31] To ensure efficiency of 1× MIC at the transcriptional level, 10× MIC was also tested, resulting in 7.3% changes. A lower number of regulated genes was also reported when five times the MIC of various drugs was added to *M. tuberculosis* in comparison with 1× MIC.[32] Dose-dependent difference in the expression patterns was observed, a point in agreement with previous reports.[33] Altogether, these data suggest that the effects observed with 1× MIC are specific.[34] Analysis of transcripts detailed below further revealed that incubation of *T. whipplei* for 6 h with 1× MIC doxycycline resulted in transcriptional changes that fit well with the characteristic signature of translational inhibitors and that mainly correspond to the direct expected effects.[31] In contrast, the 10× MIC exposure generates changes related to the resistance mechanisms considered as indirect effects.[31]

Absence of heat-shock response

The heat-shock response, also considered as the universal stress response, is often induced in response to various antibiotics.[31] In this study, heat-shock proteins were not modified when *T. whipplei* was exposed to doxycycline, as observed for *Mycobacterium tuberculosis* treatment with translation-targeted antibiotics including tetracycline or roxithromycin.[30] Using a bi-dimensional gel analysis, the response of *Escherichia coli* to tetracycline was shown to be associated with the cold-shock response.[35] Here again, we failed to evidence significant homologies with what was observed when *T. whipplei* was incubated at cold temperatures, a response mainly characterized by GroEL2 and ClpP1 up-regulation.[21] These results reinforce the uniqueness of the adaptive response of *T. whipplei* to the thermal stress[21] and favour the hypothesis that this bacterium possesses still unknown regulation pathways.

Expected effects of doxycycline on protein synthesis

Consistent with the inhibitory effect of doxycycline on translation,[31] exposure of *T. whipplei* to the MIC of this antibiotic was mainly characterized by the up-regulation of several genes that encode ribosomal proteins (*rplM*, *rpsT*, *rpsG* and *rpsL*). Such an increase in ribosome production capacity is aimed at restoring protein synthesis and is generally accompanied by the concomitant increase in rRNA transcription.[34] Therefore, under our experimental conditions, both *rpoA* and *rpoB* were down-regulated, reinforcing the defect of protein synthesis in response to doxycycline.

Doxycycline impact on T. whipplei replication

Analysis of our data revealed that proteins involved in DNA replication and repair were significantly down-regulated. They included DNA gyrase, a type IV topoisomerase (*parC*) [EC:5.99.1.-] that alters DNA topology,[36] and the superfamily I DNA helicase (*pcrA*) [EC:3.6.1.-] that favours an opening up of the DNA helix. The latter is an enzyme found in Gram-positive bacteria that exhibits a primary structure highly homologous to the Rep, UvrD and RecB(CD) helicases of *E. coli*.[37] The DNA repair ensured by the Ogt alkyltransferase encoded by *ogt*[38] was found to be down-regulated, which is consistent with the up-regulation of *ruvC* that endonucleolytically resolves Holliday junctions. These structures are formed as transient DNA intermediates during site-specific and homologous recombination and play a critical role in normal DNA metabolism and repair.[39] As a consequence, their dissociation should result in an altered bacterial growth. The concomitant up-regulation of the DNA ligase (*ligA*) [EC:6.5.1.2], another major actor of replication machinery, is more intriguing.

ParA- and ParB-like proteins, which are components of the chromosomal segregation machinery of several bacterial species[40] located in the same cluster within the *T. whipplei* genome,[16] were found to be overexpressed following doxycycline treatment. The overexpression of ParA is expected to repress the transcription of both genes from a promoter upstream of *parA*.[41] As a consequence, this should slow down the separation of newly replicated chromosomes towards the cell poles.

Two essential cell division proteins located at the septum site in *E. coli*, namely FtsX and FtsQ,[42] were also negatively regulated. Collectively, these data support a reduced growth rate of *T. whipplei* treated with doxycycline.

Indirect metabolic changes induced by doxycycline

Exposure of *T. whipplei* to doxycycline impacted the fatty acid metabolism, as shown by the decreased expression of *kasA*. The *kas* operon is implicated in the synthesis of mycolic acid biosynthesis, one of the major components of mycobacterial cell walls[43] and is modulated by various drugs.[32] KasA was recently demonstrated as an essential component of the fatty acid synthase II machinery in *Mycobacterium smegmatis*.[44] The authors hypothesized that the cell lysis observed in deletion mutants was not necessarily the direct consequence of cell wall damage but should result from indirect changes in cellular metabolic processes mediated by KasA.

Analysis of obtained data also evidenced the down-regulation of 1-deoxy-D-xylulose 5-phosphate reductoisomerase (*dxr*) [EC:1.1.1.267]. This enzyme belongs to the methylerythritol phosphate pathway to isoprenoids, a mevalonate-independent alternate biosynthetic route found in many pathogenic bacteria.[45] This pathway is catalysed by IspDF, a bifunctional enzyme that exhibited a lower transcription upon doxycycline treatment, a point in accordance with the decrease in *dxr* transcripts. Both enzymes have been described as attractive targets for the development of new antibacterial compounds.[45,46] Changes of genes related to carbohydrate metabolism were essentially observed with 10× MIC doxycycline. We believe that such a response is an indirect effect aimed to compensate for the altered growth of *T. whipplei* in the presence of this antibiotic.

Mechanism-related changes in gene transcription involve the ppGpp alarmone

A variety of transcription attenuation mechanisms are used by bacteria to regulate gene and operon expression.[47] In this respect, we noticed the up-regulation of the gene encoding the tryptophanyl-tRNA synthetase (*trpS*). This enzyme which favours incorporation of tryptophan into proteins has long been known to regulate the termination of transcription at the attenuator site between the operator and the structural genes of the tryptophan operon.[48] Only one of the five structural genes classically described within the *trp* operon[49] was evidenced within the *T. whipplei* genome, namely the *trpE* gene that converts chorismic acid into tryptophan.[16] We noticed that the upstream pathway of chorismate biosynthesis was altered by the 10× MIC doxycycline. Under those conditions, a down-regulation of the shikimate kinase (*aroK*) [EC:2.7.1.71] was indeed observed, leading us to hypothesize that the tryptophan pool is reduced in *T. whipplei* exposed to doxycycline. It is known that tryptophan starvation induces the accumulation of the regulatory nucleotide ppGpp, which is synthesized from GTP by the *relA* gene product, ppGpp synthetase I.[50] This is in agreement with the observed up-regulation of *relA* [EC:2.7.6.5]. Moreover, an increase in purine metabolism, a feature commonly observed with translation inhibitors,[29,33] was also observed. Altogether, and as previously evidenced for *Haemophilus influenzae*,[51] the *T. whipplei* response to translational inhibitors is likely to be mediated by ppGpp synthesis.

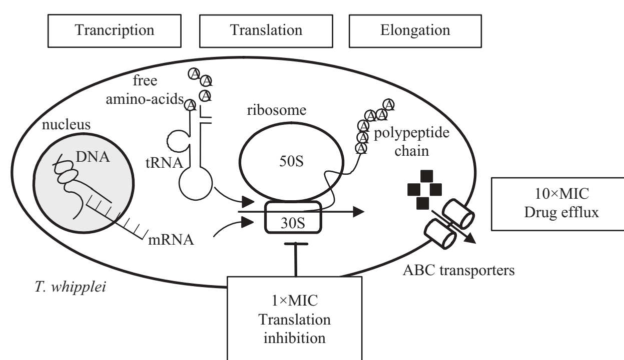

Figure 4. Schematic representation of the bacteriostatic activity of doxycycline. The analysis of the transcriptional profiles of *T. whipplei* exposed to doxycycline (1× MIC) revealed that this antibiotic inhibits the protein synthesis by targeting genes involved in translation. The observed up-regulation of ABC transporters in the presence of an increased concentration of this antibiotic (10× MIC) is believed to modulate its accumulation within bacteria. Filled black squares illustrate doxycycline molecules.

Identification of an ABC transporter regulon

While the association of doxycycline with hydroxychloroquine was able to kill *T. whipplei*, when used alone, the former only displays a bacteriostatic activity.[12] In order to resist the toxic effects of antibiotics, microorganisms have developed various mechanisms including active efflux. Thus, multidrug efflux pumps, which include the ATP-binding cassette (ABC) multidrug transporters, can mediate extrusion of a wide variety of structurally unrelated antimicrobial agents.[52] The capacity of ABC transporters to extrude doxycycline has been demonstrated.[53] As several genes encoding for such transporters are induced in response to 10× MIC doxycycline exposure, it is possible that they serve to extrude this antibiotic from the bacteria, and hence favour detoxification as schematically illustrated in Figure 4.

While it has long been believed that the mechanisms of action of some antibiotics were well understood, recent whole-genome transcriptional analysis contradicts this notion.[25] The present analysis of the *T. whipplei* transcriptome in response to doxycycline confirms the complexity of such a response, masked in part by the unknown nature of the majority of modified targets. Nevertheless, we believe that this approach may be useful to further delineate the susceptibility of *T. whipplei* to antibiotics.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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